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Bacterioplankton Production in the Western Mississippi Sound and the Role of Photosynthetic Extracellular Release as a Driver of Bacterial Production

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BACTERIOPLANKTON PRODUCTION IN THE WESTERN MISSISSIPPI SOUND AND THE ROLE OF PHOTOSYNTHETIC EXTRACELLULAR RELEASE AS A DRIVER OF BACTERIAL PRODUCTION

by

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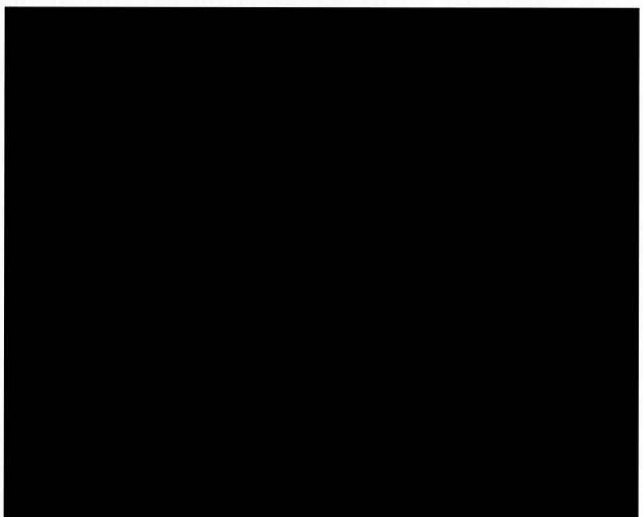

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ABSTRACT

BACTERIOPLANKTON PRODUCTION IN THE WESTERN MISSISSIPPI SOUND AND THE ROLE OF PHOTOSYNTHETIC EXTRACELLULAR RELEASE AS A DRIVER OF BACTERIAL PRODUCTION

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Bacterioplankton growth and metabolic activities are a vital component in structuring marine and estuarine ecosystems. In this study, bacterial production (BP) was investigated at two separate stations in the western Mississippi Sound (MS). One was an inshore site outside of the Bay of St. Louis and the other site was located in the transition zone (offshore) between the MS and Mississippi Bight, just south of Cat Island. Bacterial production (BP) was analyzed using the addition of ^{14}C leucine to natural water samples. In addition, samples were augmented with algal exudates in order to investigate BP based on uptake of extracellular organic carbon released by a diatom (*Chatoceros gracile*) and a chlorophyte (*Oocystis sp.*). The addition of diatom photosynthetic extracellular release (PER) versus chlorophyte PER was assessed to see the affect on BP rates as well. Bacterioplankton production was measured using the leucine incorporation microcentrifuge procedure (Kirchman 2001). These analyses were conducted from July 2011 through December 2011. The highest bacterioplankton production rates were found in July and lowest in December, with BP values ranging from 14 $\mu g\ C\ l^{-1}\ d^{-1}$ to 425 $\mu g\ C\ l^{-1}\ d^{-1}$. There was no significant difference between bacterial production rates between stations but a strong seasonality was found between summer and fall months. Spearman's rank analysis indicated that BP was correlated

positively with chl *a*, *in situ* temperature, and silicate, but no significant correlation was found with inorganic nutrients. There were no significant differences between PER enhanced BP rates and non-augmented BP. In addition, there were no significant differences in BP when enhanced by diatom or chlorophyte PER. These results suggested that BP in the western Mississippi Sound did not appear to be limited by inorganic nutrients and labile dissolved organic matter (DOM). Phytoplankton were found to be an important labile substrate for bacterial production in this region. Based on the high BP results, heterotrophic bacteria may be of greater trophic importance than what was thought before in the western MS.

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LIST OF ABBREVIATIONS

Ammonium	NH_4
Bacterioplankton Production	BP
Colored Dissolved Organic Matter	CDOM
Chlorophyll a	chl *a*
Dissolved Inorganic Nitrogen	DIN
Dissolved Organic Matter	DOM
Dissolved Organic Carbon	DOC
Kolmogorov-Smirnov	KS
Kruskall-Wallis analysis	KW
Leucine	Leu
Mann-Whitney test	MW
Mississippi Sound	MS
Principle Components Analysis	PCA
Photosynthetic Extracellular Release	PER
Phosphate	PO_4
National Climate Data Center	NCDC
National Data Buoy Center	NDBC
Nitrate	NO_3
Silicate	$Si(OH)_4$
Thymidine	Tdr
Total dissolved nitrogen	TDN

Trichloroacetic acid	TCA
United States Geological Survey	USGS
Windspeed	WSPD
Wolf River	WR

CHAPTER I

INTRODUCTION

Bacterioplankton growth and metabolic activities are a vital component in structuring aquatic ecosystems. Pelagic marine bacteria can remineralize complex compounds and nutrients to be made available for primary production. They also act as a pathway of organic matter and nutrients to higher trophic levels (Sherr et al. 1988). Increased information and knowledge about the linkages between organic carbon delivery and its microbial decomposition and cycling are critical for marine ecosystems (Azam et al. 1983).

It has been found in scientific studies of phytoplankton, bacterioplankton abundance and production that there are significant correlations between bacterial and phytoplankton processes in marine waters. Kirchman and Hoch (1988) found that when phytoplankton biomass varies, bacterial production varies simultaneously. Phytoplankton production has been found to be an important determinant of variation in bacterial production. This has suggested a relationship between bacterioplankton and phytoplankton. These relationships have supported a general conceptual model of bacterial dependence, either direct or indirect on algal production as a primary source of organic substrate. The main purpose of this study was to examine bacterioplankton production and interactions in the Mississippi Sound (MS) as well as the role phytoplankton exudate may play in stimulating bacterioplankton production.

Phytoplankton are not only an important source of autochthonous particulate organic matter, but these organisms contribute to the production of dissolved organic matter (DOM). Up to 50% of photosynthetically fixed carbon is released in the form of dissolved organic carbon (DOC; Azam et al. 1983). The fate of the newly produced

DOC in the marine environment is mainly determined by bacterial uptake. Bacterial biomass in the oceans ranges between 10 to 40% of phytoplankton biomass (Ducklow 1983, Gundersen et al. 2001). This release of extracellular organic carbon serves as an energy source for bacterioplankton (Jensen 1983).

Coastal and estuarine systems are sites where competition for nutrients by microorganisms and remineralization of photosynthetically fixed carbon are important processes (Wheeler and Kirchman 1986). The MS is a shallow eutrophic coastal environment that is seen as a complex system with the addition of increased organic matter, nutrients, and other anthropogenic substrates coming from surrounding bays, rivers and bayous. Investigating bacterial production and its controlling factors will help in developing a better understanding about the dynamics of the carbon cycle in this coastal environment.

In many estuarine systems, the majority of autochthonous carbon is derived from phytoplankton. This DOC is considered to be the most labile carbon source (Bronk et al. 2006). Bacterial growth efficiencies have been found to increase as the substrate becomes more labile (Coffin et al. 1993). Therefore, phytoplankton extracellular release has the ability to influence the resultant productivity of bacterial populations utilizing the DOM excreted. Measuring bacterial production allows bacterial responses to ecological conditions to be assessed. The main objective of this study was to investigate the growth of a bacterioplankton community near the Mississippi coast and bacterial growth at the transition zone between the MS and the Mississippi Bight. There was also an experiment involving the addition of algal exudates to water samples in order to investigate bacterial

production based on uptake of extracellular organic carbon released by a diatom (*Chaetoceros gracile*) and a chlorophyte (*Oocystis sp.*).

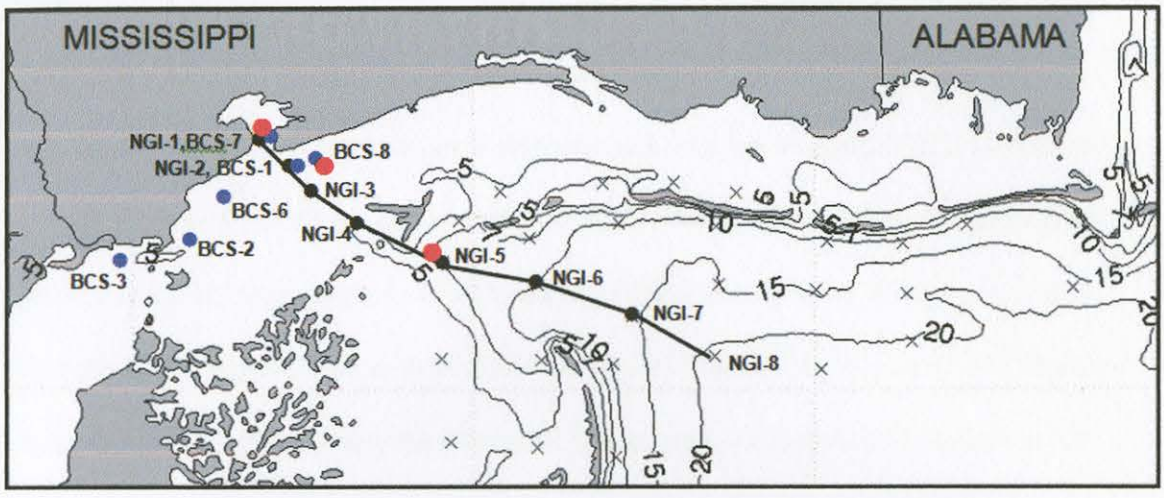


Figure 1. Map of station locations on BSC and NGI sampling transects. The two sampling stations used for this project were BCS 8 and NGI 5.

Background

The MS is a shallow, coastal estuarine system. The length of the sound is 80 miles (120 km) along the Gulf of Mexico Coast from Mobile Bay, Alabama in the east, to Lake Borgne, Louisiana in the west (Kjerfve 1983). A series of vegetated barrier islands (Cat, Horn, Ship and Petit Bois) encloses the seaward southern border of the Sound that separates the Mississippi Sound from the open Gulf of Mexico.

This region is most often well mixed vertically by winds, but has horizontal stratification because of longitudinal salt gradients (Kjerfve 1989). Salinity distributions are highly variable both spatially and temporally, mainly due to river runoff, with typical mean salinities between 12 and 27 (Kjerve 1983). Freshwater influences include runoff directly from the Pearl River, Pascagoula River, Mobile River and to a lesser degree the Biloxi, Jourdan, and Wolf Rivers. Also, the Sound receives indirect input from the Mississippi River. The Bonnet Carre Spillway is a means for the human controlled

discharge of the Mississippi River that would provide more freshwater influence on the MS. The spillway enables the Army Corps of Engineers to control Mississippi River discharge and protect New Orleans from floods. The excess water entering into Lake Pontchartrain will exit through the Rigolets and then into the western MS. Since its completion in 1931, the Bonnet Carre spillway has only been opened 10 times and most recently on May 9th 2011 to mitigate river levels in the Mississippi River Delta region. The MS receives large amounts of nutrient input from surrounding freshwater sources. Phytoplankton biomass varies seasonally with lower biomass in the winter and higher phytoplankton biomass in late summer/fall. There has been found to be a decrease in phytoplankton abundance when moving away from the shoreline to more open water (Atwell 1973; Lohrenz and Verity 2004).

Bacteria are responsible for the major component of heterotrophic activity in most aquatic environments (Cole et al. 1988). Respiration rates and total biomass tend to be dominated by the bacterioplankton size fraction. Schwaerter et al. (1988) found that bacteria were accountable for approximately 50% of total community respiration. Heterotrophic bacteria have two major roles in the cycling of organic matter and nutrients: production of biomass and remineralization of nutrients and carbon (Coffin et al. 1993). Bacterioplankton assimilate organic matter into biomass, which is then potentially available to higher trophic levels while also releasing CO_2 that is then accessible to primary producers. Carbon released as DOM is returned to the main food chain through the microbial loop of bacteria to micrograzers (Azam et al. 1983).

Bacterial production (BP) is defined as secondary production: the synthesis of bacterial biomass, from dissolved organic matter with some inorganic nutrients

(Ducklow 2000). In a review of bacterial production, Ducklow (2000), explained that biomass production is the increase in biomass per unit time per unit volume. It is a function of both biomass (B), expressed as carbon mass per volume, and the specific growth rate (μ) expressed in units of inverse time (t). In the absence of any mortality (grazing), there will be an exponential increase in bacterial biomass, which can be expressed by Equation 1:

$$dB/dt = \mu B$$

Kirchman (2001) explained that in natural systems bacteria do not normally live in the absence of protist grazers and viruses, which cause bacterial mortality. Furthermore, bacterial production is mainly matched by mortality ($(dB/dt)_{net} = 0$). However, the present methods involving incorporation of radioisotope-labeled precursors of proteins to measure BP measure 'gross production', meaning biomass unaffected by mortality. This is possible because the incubation periods for these methods are short, about an hour or less, compared to the timescale of bacterial growth and mortality. These methods still do not include respiration although 'gross production' is being measured (Kirchman 2001).

An estimate of bacterial production can be used as a general indicator of bacterial activity (Kirchman 2001). Cole et al. (1988) studied a wide range of environments measuring bacterial production and concluded that approximately 40% of the primary production is required to support bacterial carbon demand. The BP is commonly measured indirectly using radioisotope-labeled precursors of DNA or protein to yield synthesis rates (Ducklow 2000). These rates can then be converted to production rates. An early approach using ^{3}H-adenine was the first modern method for estimating

bacterial production (Karl 1979). Presently, the most common approaches used are radioisotope-labeled thymidine and leucine (Kirchman 2001).

Karl (1979) initially measured incorporation of ^{3}H-adenine into RNA in samples from the Caribbean Sea, and studies later expanded to pure cultures of bacteria and to other oceanic environments (Karl et al. 1981; Karl and Winn 1984). Since bacteria, phytoplankton, and other microorganisms incorporate ^{3}H-adenine, Karl (1979) decided to apply the adenine technique to estimating total microbial rates. The ^{3}H-adenine is taken up rapidly by bacterioplankton, supplying a high sensitivity method. However, the uncertainty of this method is the major reason why scientists did not take up this approach when looking at heterotrophic bacterial production specifically (Ducklow 2000).

Thymidine (TdR) incorporation was introduced as a measurement of BP by Fuhrman and Azam (1980). The use of ^{3}H-TdR incorporation reflects DNA synthesis or cell production. Hollibaugh (1988) explained that the concept and methodology for TdR incorporation is simple, but the conversion of incorporation rates to growth rates is based on many assumptions. One main assumption is that the exogenous radioisotope-labeled TdR is incorporated into DNA without metabolic alteration (Fuhrman and Azam 1980, 1982). Metabolism of radiolabled TdR could be incorporated into DNA through other precursors. Thymidine metabolism could result in tritium incorporation into a variety of macromolecules in addition to DNA. If the assumptions are met, the TdR approach offers an easy protocol, specific for estimating the production rate of actively growing cells (Ducklow 2000).

Although the TdR method is still used, Kirchman (2001) believed that measuring bacterial protein synthesis by the incorporation of radioisotope-labeled leucine provided the most direct estimate of bacterial production (Kirchman 2001). The leucine (Leu) method is based on protein synthesis. Leucine is in constant proportion for all bacterial protein (7.3%) and protein is a relatively constant fraction of bacterial biomass (about 60%; Kirchman 2001). Therefore, biomass production can be calculated from rates of protein synthesis. Furthermore, Leu is not transformed to other amino acids, which would be incorporated into protein and potentially lead to overestimates of the production rate. On the other hand, there are processes that are independent of net biomass production; that can add variations in Leu incorporation and lead to errors in estimating bacterial production. Kirchman (2001) pointed out that Leu can be synthesized from other compounds, leading to isotope dilution of the radioisotope-labeled leucine. A way to minimize this problem is to add Leu in concentrations higher than *in situ* concentrations. This means that the natural extracellular Leu can be ignored and bacteria will take up the exogenous labeled Leu while repressing the production of non-radioactive leucine to be used for protein synthesis (Kirchman 2001). The Leu method is more straightforward than the TdR method. The only variable assumption in converting Leu incorporation to BP is the degree of isotope dilution (Kirchman 1993). Over recent years, Leu incorporation has been more widely used than the TdR method. (Kirchman 2000).

Ducklow (2000) reviewed studies looking at bacterial production rates ranging from open-ocean environments to estuarine coastal areas. He made a simple generalization that bacterial biomass in the euphotic zone averages about 1-2 g C m^{-2},

except in the Antarctic, where it is lower. It is also acknowledged that bacterial populations decline in size when moving off shore from estuaries toward the more oligotrophic ocean (Sieburth 1979). Ducklow (2000) stated, estimates of bacterial abundance range from 1-5 x 10^8 cells L^{-1} in most oligotrophic regions to over 2 x 10^{10} cells L^{-1} in rich estuaries. Additionally, top-down controls seem to be stronger in estuaries. Bacteria may form a more solid link to higher trophic levels inshore because large amounts of carbon are sequestered to bacterial biomass.

Murrell (2003) examined patterns of bacterioplankton metabolism in the Pensacola Bay estuary located in northwestern Florida. Bacterial production showed a seasonal pattern with a positive correlation to temperature and chl *a* measurements. Bacterial growth in Pensacola Bay averaged 115 μg C l^{-1} d^{-1}. This average is above the average of 50 to 73 μg C l^{-1} d^{-1} reported for estuaries by Ducklow and Carlson (1992). Several studies have been done in the Mississippi River plume region and open Gulf waters as well (Chin-Leo and Benner 1992; Cotner and Gardner 1993; Biddanda et al. 1994, Pakulski et al. 1995; Pomeroy et al 1995). Chin-Leo & Benner (1992) found that bacterial production ranged from 4 to 90 μg C l^{-1} d^{-1}. Cotner and Gardner (1993) found a range of 4.3 to 107 μg C l^{-1} d^{-1}, Pakulski et al. (1995) 10 to 90 μg C l^{-1} d^{-1} and Biddanda et al. (1994) 2.4 to 74 μg C l^{-1} d^{-1}. Pomeroy et al. (1995), studying at mainly oligotrophic sites, measured a range of 0.7 to 28 μg C l^{-1} d^{-1} during summer months. Looking at these results, Murrell's bacterial production rates are the highest reported from the Gulf of Mexico region. Since the MS is another warm estuarine system like the Pensacola Bay area, comparing bacterial growth results will give a better view of coastal estuarine dynamics.

Extracellular release of DOC of photosynthetic origin is not a methodological artifact, although bottle containment may be an issue, it is a real occurrence through either direct release from intact cells (Obernosterer and Herndl 1995) and/or indirect mechanisms. These indirect mechanisms include cell lysis or zooplankton grazing (sloppy feeding; Fogg et al. 1977). Cell lysis has led to high levels of released extracellular material (Granum 2002). It is well known that extracellular release is high during the stationary phase of phytoplankton growth, but it is still disputed whether a significant amount is released from actively growing cells (Sharp 1977; Mague et al. 1980; Myklestad et al. 1989). Phytoplankton have been found to generate five times more DOC in the stationary phase than the exponential phase (Myklestad et al. 1989). Bacterial uptake is the fate of the newly produced DOC in the marine environment.

Results of laboratory studies have shown that bacteria can rapidly utilize DOC from phytoplankton (Chen and Wengersky 1996). A study done by Kritzberg (2004) found that bacteria utilized DOC of recent autochthonous origin preferentially over DOC of terrestrial origin. The degree to which bacteria utilize autochthonous versus allochthonous material for growth seemed to be based on the supply, lability, and quality of the two sources (Painchaud and Therriault 1989). Allochthonous DOM has already undergone some degradation and transformation before entering the marine environment and is considered recalcitrant to bacterial utilization because of high molecular weight and aromaticity (Moran and Hodson 1994). Phytoplankton derived DOM is mainly composed of low molecular weight compounds and is more available for uptake by bacteria (Chen and Wengersky 1996).

Carpenter (2010) performed an amendment experiment using Mississippi coastal water to see if autochthonous DOM was utilized more efficiently by heterotrophic bacteria than allochthonous or marsh/terrestrial DOM. The results showed bacterial production rates of the autochthonous DOM treatment to be 72 μg C l^{-1} d^{-1} compared to the allochthonous DOM treatment of 9 μg C l^{-1} d^{-1} day. In a study done by Chin-Leo and Benner (1992), bacterial production and abundance were measured during summer and winter across the salinity gradient of the Mississippi River plume, in oligotrophic waters of the Gulf of Mexico, and at sites within the river. During both seasons, bacterial production in surface waters was enhanced at intermediate salinities (15 to 30%). This area was also where the highest phytoplankton biomass was found. These results suggested that phytoplankton growth was the main driver in stimulating bacterial production.

The studies referenced above indicate that phytoplankton derived DOC is an important food source for bacterioplankton. Diatoms and chlorophytes are found regularly in and near coastal waters of the Gulf of Mexico. Molina (2011) studied phytoplankton abundance in western MS waters. She found that diatoms predominated during all seasons and were more important during the summer months. Chlorophyte values were found to be different between seasons with highest values associated with the summer months. Diatoms (Bacillariophyceae) can be characterized as the largest of the phytoplankton and comprise the most abundant marine phytoplankton species (Granum 2002). Chlorophytes, such as *Oocystis sp.*, are green unicellular microalgae found normally singular or as a colony (Protist information server 2010 http://protist.i.hosei.ac.jp/). Different species of phytoplankton have shown dramatic

differences in dissolved organic carbon (DOC) excretion. For example, diatoms in the genus *Chaetoceros* appear to have high extracellular production (20 to 50% of total photosynthetic production) in general while *Thalasiosira spp.* and *Skeletonema costatum* produce less (1 to 7% of total photosynthetic production; Granum et al. 2002). The dominant algal species and the physiological state of the phytoplankton seem to influence strongly the amount of DOM excreted. The rates of this DOC uptake by bacterioplankton depend also on the physiological state of the bacterioplankton and the chemical composition of the organic substrate (Puddu et al. 2003).

Significance

Presently, no study has been performed to look at bacterial utilization of DOM in the western MS region. This area is a very complex system and considering the potential importance of allochthonous carbon inputs in estuaries, bacterial production may be relatively high. Therefore, bacteria, both in terms of production and carbon flux, might have a greater trophic importance in estuarine than in either freshwater or marine ecosystems (Painchaud et al. 1989). Bacterial production data for the Gulf of Mexico region are underrepresented in literature, especially in coastal estuarine environments such as the MS. Existing literature has mainly focused on the very productive Mississippi River plume region as well as oligotrophic Gulf sites (Murrell 2003; Chin-Leo & Benner 1992; Cotner & Gardner 1993; Biddanda et al. 1994; Pakulski et al. 1995; Pomeroy et al. 1995). Studying bacterial production or stimulation by DOM can provide important information about the dynamics of the carbon cycle in this warm, subtropical system.

From culture studies, carbohydrates are reported as the major components of phytoplankton exudates, but their molecular composition is highly variable depending on species and nutrient status (Puddu et al. 2003). This variability can have considerable effects on microbial ecosystem dynamics. Also, the effects on bacterial communities can provide potential feedback to other phytoplankton, including harmful algal species (Pete et al. 2010). The results of this study can provide further insight into the regulating mechanisms phytoplankton play in the microbial loop.

Bacterioplankton species are used as indicators for assessing water quality. Exploring bacterial production rates near the coast and at the transition zone between the MS and open Gulf water can offer useful information for environmental managers (Kjerve 1983). Learning about the effects that different water conditions have on bacterial production could be crucial because the MS is a very complex area and changes in phytoplankton and microbial community structure in relation to the composition and level of nutrient and DOM input, may, in turn, lead to altered food web structure. This impact on food web structure could have effects on the surrounding environment. Since the Sound is of great economic importance for fisheries and other recreational activities it would be beneficial to continue studying this valuable coastal zone (Lohrenz and Verity 2004).

Objective

The main objective of this study was to investigate bacterial production at two separate sites in the western MS. One was a coastal site outside of the Bay of Saint Louis and the other site was located in the transition zone between the MS and the Mississippi Bight, just south of Cat Island. Another objective was to perform an experiment

involving the addition of algal exudates to water samples in order to investigate if bacterial production was stimulated from the based on uptake of extracellular organic carbon released by a diatom (*Chaetoceros gracile*) or a chlorophyte (*Oocystis* sp.*)* A final objective for this project was to assess if the addition of diatom (*Chaetoceros gracile*) versus chlorophyte (*Oosystis* sp.*)* PER would affect bacterial production rates differently or similarly.

Hypotheses

1. Bacterial production will be higher at the inshore station (BCS 8; 30.298 N, -89.263 W) compared to bacterial production rates found at the offshore station (NGI 5; 30.161 N, -89.045 W).
2. Water samples that are augmented with PER will have higher bacterial production compared to non-augmented water samples.
3. Water samples augmented with diatom PER will have higher bacterial production rates compared to water samples augmented with chlorophyte PER.

CHAPTER II

MATERIALS AND METHODS

To test the hypotheses, samples were collected in the western MS monthly, from July 2011 through December 2011. The monthly cruises utilized the USM research vessel, *Lemoyne.* Sampling and data analyses were conducted through Northern Gulf Institute (NGI) and the Coastal Impact Assistance Program (CIAP).

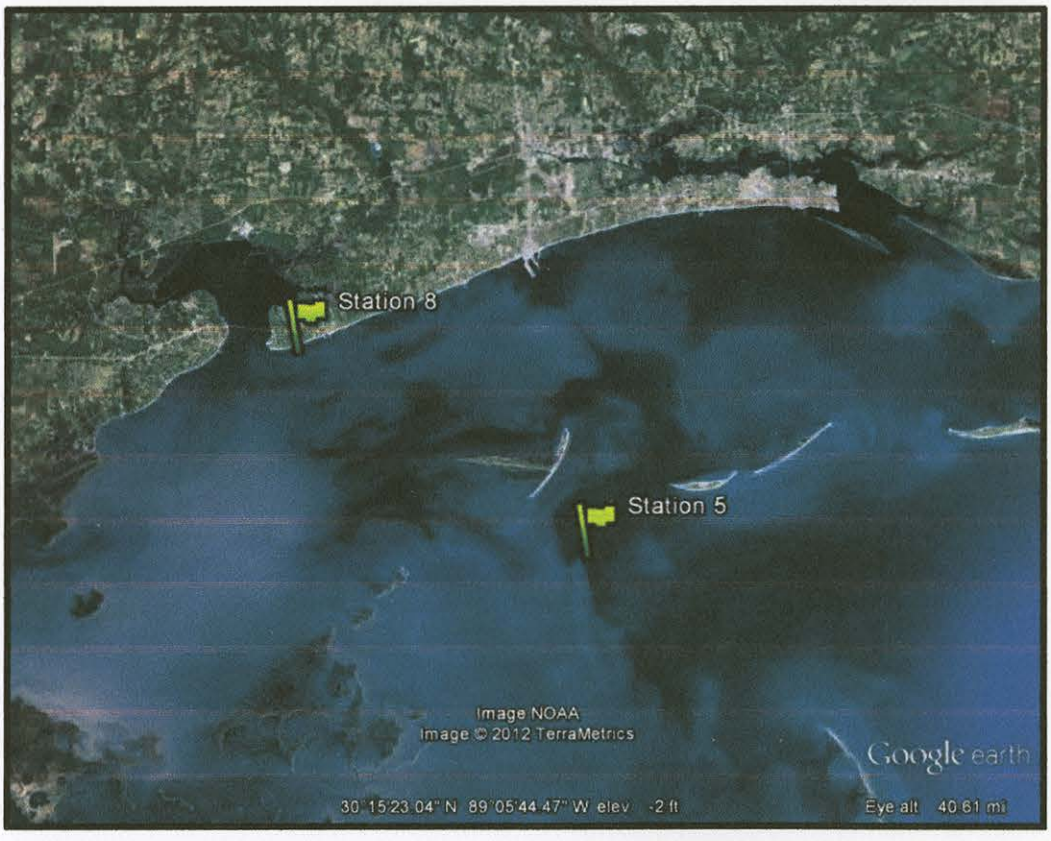


Figure 2. Study area map for western MS. Coordinates of stations are given in Appendix D. Map made in Google Earth.

BCS 8 is located south of Pass Christian Harbor to the east of Bay St. Louis. This location is an area with multiple river discharge, contributing to different levels of nutrient and DOM input. NGI 5 is located southeast of Cat Island and southwest of Ship Island in the transition zone between the MS and Mississippi Bight. This station is

located in a very dynamic area that is somewhat removed from the influences of freshwater with increasing salinity and changes in nutrients/DOM compared to BCS 8 coastal site. Water samples were collected in triplicate at each station in 150 mL acid-washed HD polyethylene bottles and stored in a cooler at ambient temperature until laboratory analysis.

Experimental Procedure

As soon as samples were returned to the laboratory, three subsamples from each triplicate bottle were augmented with diatom PER and another three-subsample set was augmented with chlorophyte PER. A final three subsamples were taken from each triplicate sample and were not augmented with phytoplankton PER. All sets were inoculated with ^{14}C leucine and followed the leucine incorporation microcentrifuge procedure (Smith and Azam 1992, Kirchman 2001) to determine bacterial production for each set of samples. This procedure was followed for both BCS 8 and NGI 5.

PER Inoculum

Cultures of *C. gracile* and *Oocystis sp.* were grown in FCRG medium (see Appendix E) and incubated in a Sanyo Versatile Environmental Chamber at 25°C with light levels at 200 µmol quanta m^{-2} s^{-1} under a 12:12 light cycle. Growth was monitored using fluorescence measurements and cell counts. Once growth reached the stationary phase, phytoplankton were removed from the medium by filtration using 0.8 µm Nucleopore polycarbonate filters. After phytoplankton filtration, bacteria were removed from medium using 0.2 µm Nucleopore polycarbonate filters. Filtrate was then frozen in separate tubes with one tube from each phytoplankton species thawed out the day before sampling for use in experiments for that sampling day. This was to ensure all

experiments were getting the same filtrate PER from each phytoplankton species. This also ensured that the PER generated from each phytoplankton species was from cultures at the same physiological state throughout the project.

Laboratory Analyses

In vivo fluorescence for the diatom and chlorophyte phytoplankton species was determined using a calibrated Turner Designs Model 10 AU fluorometer equipped with the Welschmeyer filter set to monitor phytoplankton growth (Turner Designs algal pigments method S-0053). Cell numbers were determined using a Beckman-Coulter Z2 Particle and Size Analyzer (Beckman Coulter Z series User Manual 1997).

Chlorophyll *a* Analysis

Fluorometric analysis of chlorophyll samples were also conducted using the Turner Designs Model 10 AU fluorometer. Cells were collected on a 2.5 cm diameter Whatman GF/F filter (0.7 *u*m-nominal pore size) under low vacuum (≤100 mm Hg). The filters were then placed in glass borosilicate tubes containing 6 mL of 100% methanol. The borosilicate tubes were placed in the freezer (-4°C) for 24 hours to allow for pigment extraction in the dark. The tubes were removed from freezer, thawed and then vortexed for 10 seconds. The filters were removed from the methanol and discarded. Each tube was centrifuged for 10 minutes and placed in the fluorometer to be read. Chlorophyll samples were read three times and an average value was calculated. An additional blank containing pure methanol was read to correct the values. Chlorophyll *a* concentration was calculated by the following equation:

$$\text{Chlorophyll } a \text{ (µg/L)} : [(\text{F-blank})*(6 \text{ mL/V})*10^3]/912.57$$

Where F is the fluorometric value read from the fluorometer and V is the volume of sample filtered.

Nutrient Analysis

Nutrient samples were pre-filtered through a 2.5 cm diameter Whatman GF/F filter (0.7 *u*m-nominal pore size) at low vacuum (≤100 mm Hg). The filtrate was collected in an acid washed flask, stored in a clean acid rinsed (10% HCL) 125 mL HD polyethylene bottle, and frozen (4°C) until time of analysis. The samples were returned to room temperature for analysis and all nutrients were measured using fluorometric (N species) and spectrophotometric (PO_4 and $Si(OH)_4$ methods using an Astoria-Pacific A2+2 nutrient analyzer (Method #A179, A027, A205, and A221; Astoria-Pacific International, Oregon USA).

DOC Analysis

Samples for DOC were taken by filtering water through pre-combusted Whatman GF/F filters, and the acidified (concentrated HCl) filtrate was analyzed using a Shimadzu TOC-V analyzer (Combustion catalytic oxidation/NDIR method).

Bacterial Production Procedure

Bacterial production was analyzed using the ^{14}C leucine incorporation microcentrifuge method according to Kirchman (2001). The ^{14}C leucine primary stock had a specific activity of 334 mCi $mmol^{-1}$. A final leucine concentration of 60 nM was added to each sample based on a ^{14}C leucine saturation curve calculated for both stations to avoid the problem of isotope dilution. All experiments consisted of a 30-minute incubation time.

Four 2 mL microcentrifuge tubes were prepared per sample (triplicate samples plus 1 killed control) in the laminar flow hood. To all empty tubes, ^{14}C-Leu working stock was added to give a final leucine concentration of 60 nM in the 1.5 mL seawater

sample. For a killed control in each sample set, 75 µL of 100% Trichloroacetic acid (TCA) was added to one tube. An inoculum of 1.5 mL of water sample was added to each tube. The tubes were closed, inverted and incubated in the dark at *in situ* temperature from when the samples were taken. For the augumented samples, 100 µL of diatom or chlorophyte PER was added to the microcentrifuge tube, then 1.5 mL of water sample was added. The final DOC concentration added was 2,050 µM for diatom PER addition and 2,375 µM for chlorophyte PER addition. Samples were incubated for 30-minutes. Incubations were stopped by adding 75 µL of 100% TCA to all live tubes (all triplicates) and mixed. Tubes were left to sit for five minutes. After five minutes, tubes were centrifuged at 13,000 fcr for 15 minutes using Fisher Scientific accuSpin Micro 17 microcentrifuge. Liquid was poured off from each tube into a waste container. The tube was tapped several times on drying paper to get residual drops out. Next, 1.5 mL of cold 5% TCA was added to each of the tubes and the tubes were then inverted. Tubes were centrifuged at 13,000 rcf for five minutes. Liquid was poured off as described above. After that step was completed, 1.5 mL of 70% ethanol was added and the tubes were centrifuged for an additional five minutes. Liquid was poured off as described above. Samples were left in fume hood covered loosely with paper towel for a few hours to allow any residual ethanol droplets to evaporate. Finally, there was a two mL addition of Fisher Scintisafe Plus scintillation cocktail to each tube. After 24 hours, tubes were placed in a 20 mL carrier vial and counted in a Hidex 300 SL scintillation counter.

The dpm remaining in each tube represents the leucine incorporated into cellular macromolecules. Leucine incorporation was calculated using the following equation:

Leu incorp = {(dpm on sample) – (dpm in killed control)}/incubation time/$(2.22*10^6$

dpm per μCi) * (Leu specific activity as nmol per μCi)

Bacterial production was calculated as:

Biomass production = Leu incorp * 1.5 kg C per mol

Environmental Data

Water column characteristics were obtained from *in situ* casts deployed during sampling times. Colored dissolved organic matter (CDOM) was obtained from a Wetlabs ECO FL3 optics package. Dissolved oxygen was obtained from a Seabird Electronics SB43 sensor. Temperature and salinity were acquired from the SBE CTD sensor. All data was organized in excel files to be used for statistical analysis.

Hydrologic and Weather Data

Air temperature and wind speed were downloaded from the National Data Buoy Center (NDBC 2012). The Bay Waveland Yacht Club Buoy was used for BCS 8 and the Gulfport Outer Range Buoy was used for NGI 5. Precipitation data was collected from National Climatic Data Center (NCDC) at NOAA's National Environmental Satellite, Data, and Informational Service (NCDC 2012). The Wolf River (WR) USGS gauge was used for tide gauge and discharge data (USGS 2012).

Data Analysis

Statistical analyses were conducted using SPSS® 16.0 Statistical Software (SPSS Inc.®). All non-parametric statistical tests were used based on the Kolmogorov-Smirnov test indicating that the data did not conform to the assumption of normal distribution (≤ 0.05). Kruskall-Wallis (KW), Mann Whitney (MW) rank sum, and Spearman's Rank analyses were used. A principle component analysis (PCA) was also utilized.

Figure 3. Locations of USGS WR gauge and NDBC weather stations used. Purple flag represents USGS Wolf River, blue flag represents NDBC BWYC, and yellow flag represents NDBC GPT Outer Range. For coordinates, refer to Appendix E. Image created with Google Earth.

CHAPTER III

RESULTS

BP rates were not found to vary spatially, but were found to vary temporally between summer (July, August, September) and fall seasons (October, November, December; Figure 4). There were no significant differences between algal enhanced BP rates and non-augmented BP. There were also no significant differences between BP rates between the addition of diatom PER versus chlorophyte PER (Figure 11). Non-parametric statistical analyses were used to test project hypotheses since variables were not distributed normally ($p \leq 0.05$) based on the Kolmogorov-Smirnov (KS) test. The MW was applied to determine if the data varied spatially and temporally. Kruskall-Wallis analysis was utilized to test between BP treatments. Spearman's Rank correlation analysis was used to compare BP to other biotic and abiotic variables measured. Principal component analysis was run to further determine how variables were related to one another. Twenty-one variables were measured to determine relationships between environmental parameters and BP rates. *In situ* parameters: salinity, water temperature, DO, and CDOM were measured directly in the field. Water samples were taken back to the lab and analyzed for inorganic nutrients (NO_x, NH_4, PO_4, $Si(OH)_4$), TDN, and organics (DOC and chl *a*). Weather data (air temperature, wind speed, precipitation) and hydrologic data (river discharge and river gauge height) were collected from local environmental agencies. Table 1 shows the median and range for the entire dataset.

Table 1

Median and range for the entire data set. For bacterial production, environmental parameters, inorganic nutrients, chl a and organic matter, N=12, with the exception of respiration (N=11). Data for weather and hydrologic data are by sample day (N=12).

Parameter	Unit	Median	Minimum	Maximum
1. Bacterial Production (BP)	$\mu g\ C\ L^{-1}\ d^{-1}$	91.73	14.80	425.42
2. DIN	µM	0.99	0.23	2.54
3. Ammonium (NH_4)	µM	0.85	0.05	1.89
4. Nitrate (NO_3)	µM	0.20	0.03	0.40
5. Nitrite (NO_2)	µM	0.03	0.02	0.25
6. Silicate ($Si(OH)_4$)	µM	41.84	0.94	96.00
7. Phosphate (PO_4)	µM	0.57	0.04	2.05
8. In situ Temp	°C	25.20	14.20	30.58
9. Salinity	unitless	25.92	13.78	31.78
10. Dissolved Oxygen	$mg\ L^{-1}$	6.809	4.27	14.18
11. CDOM	ppm	29.91	12.54	56.52
12. DOC	ppm	3.189	1.98	5.75
13. TDN	ppm	0.25	0.17	0.49
14. Chl a	$\mu g\ L^{-1}$	11.33	4.02	109.33
15. Air Temp	°C	23.96	10.47	29.50
16. Wind Speed	$m\ s^{-1}$	3.57	2.23	5.78
17. Rainfall	in	0.27	0.10	4.49
18. WR Gauge Height	m	1.47	1.33	1.75
19. WR Discharge	$m^3\ s^{-1}$	2.85	1.07	6.97

Bacterial Production

Highest BP rates were found in July (NGI 5) and lowest in December (BCS 8), with BP values ranging from 14 µg C l^{-1} d^{-1} to 425 µg C l^{-1} d^{-1}(Figure 4). There was no significant difference in bacterial production rates between stations ($p>0.05$; Table 3.2). The BP range at BCS 8 was 14.80 µg C l^{-1} d^{-1} (December) to 354.02 µg C l^{-1} d^{-}

^{1}(July). The range at NGI 5 was 33.34 µg C l^{-1} d^{-1} (December) to 425.42 µg C l^{-1} d^{-1} (July). Another MW was run to look at differences between the summer (July, August, September) and fall (October, November, December) season. There was a significant difference in BP between seasons ($p<0.01$) indicating a strong seasonality between summer and fall months (Table 3). This seasonality was also reflected by the strong positive correlation between temperature and BP (Figure 5).

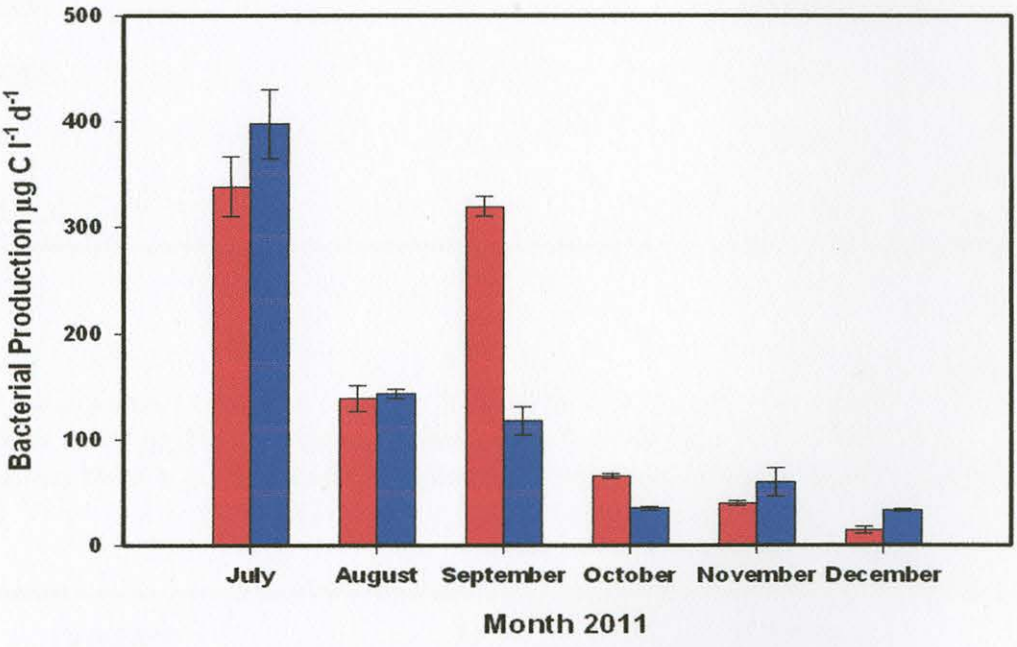


Figure 4. BP between stations throughout the sampling period. Red bars indicate BCS 8 and blue bars indicate NGI 5 BP values. The range was from 14.80 µg C l^{-1} d^{-1} (December, BCS 8) to 425.42 µg C l^{-1} d^{-1} (July, NGI 5).

Spearman's rank analysis was run to determine significant correlations between variables. Bacterial production was correlated with chl *a*, *in situ* and air temperature, $Si(OH)_4$, and precipitation (Table 4). Wind speed was correlated negatively with BP (Table 4). Chlorophyll *a* was measured as a biological parameter,

Table 2

MW test for differences between stations. N=12. The MW statistic (U) with a significance listed ($p \leq 0.01$ for values in bold, $p \leq 0.05$ for values in standard type) indicates that there is a statistically significant difference for that parameter between seasons. Dashed lines indicate no significant difference between stations.

Parameter	U	Significance
1. BP	17	---
2. chl a	4	0.025
3. Salinity	5	0.037
4. CDOM	2	0.018
5. DOC	2	0.01
6. TDN	1	0.006

Table 3

*MW test for differences between seasons. N=12. The MW statistic (U) with a significance listed ($p \leq 0.01$ for values in **bold**, $p \leq 0.05$ for values in standard type) indicates that there is a statistically significant difference for that parameter between seasons.*

Parameter	U	Significance
1. BP	0.000	0.004
2. $Si(OH)_4$	4.000	0.025
3. In situ T	0.000	0.004

Table 4

*Spearman's Rank correlation for parameters correlated significantly with BP rates ($p \leq 0.01$ for values in **bold**, $p \leq 0.05$ for values in standard type).*

	BP	chl *a*	$Si(OH)_4$	*In situ* temp.	Air temp.	5 day total precip.	Density	WSPD
BP		**0.673**	**0.727**	**0.867**	**0.790**	0.723	-0.650	-0.650

a representative of phytoplankton concentration. Chlorophyll *a* did not vary temporally, but it was found to differ significantly between stations (Table 2). Chlorophyll *a* concentrations were higher at BCS 8 compared to NGI 5 throughout the sampling period (Figure 6). Concentrations of chl *a* varied from 4.02 $\mu g\ l^{-1}$ to 109.33 $\mu g\ l^{-1}$, generally being higher during summer months. Although chl *a* concentrations differed significantly between stations, BP did not, indicating that phytoplankton biomass and processes were sufficient enough to meet BP needs in terms of DOM supply at both sites.

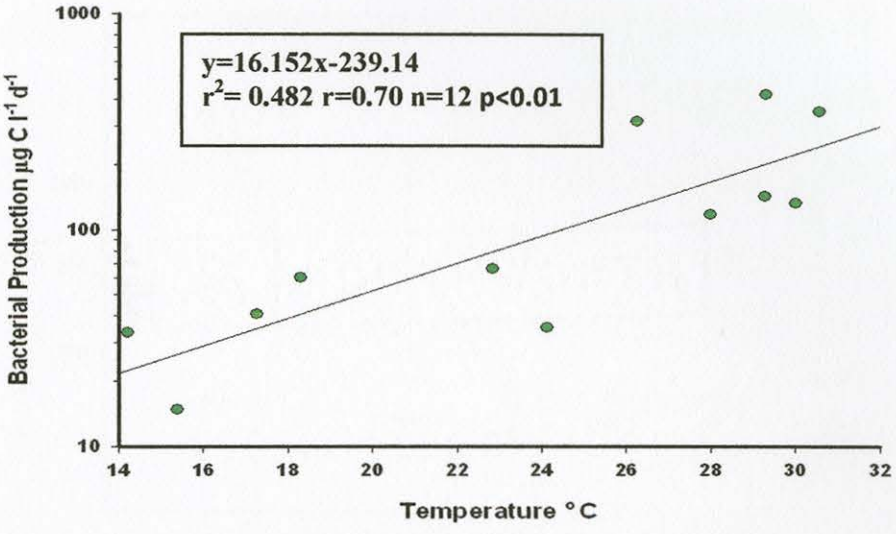


Figure 5. BP and *in situ* temperature linear regression. This relationship demonstrates the increase in BP as temperature increases. Using Spearman's rank correlation analysis was significantly correlated with *in situ* temperature ($p<0.01$).

The only dissolved inorganic nutrient found to be correlated significantly with BP was $Si(OH)_4$. Silicate demonstrated a very large range of values, from 0.940 µM to 96.00 µM, with highest values at BCS 8 in September. Multiple variables were also correlated significantly with silicate: chl *a*, *in situ* temperature, CDOM, TDN, rainfall, and WR gauge height. There was a negative correlation between silicate, salinity.

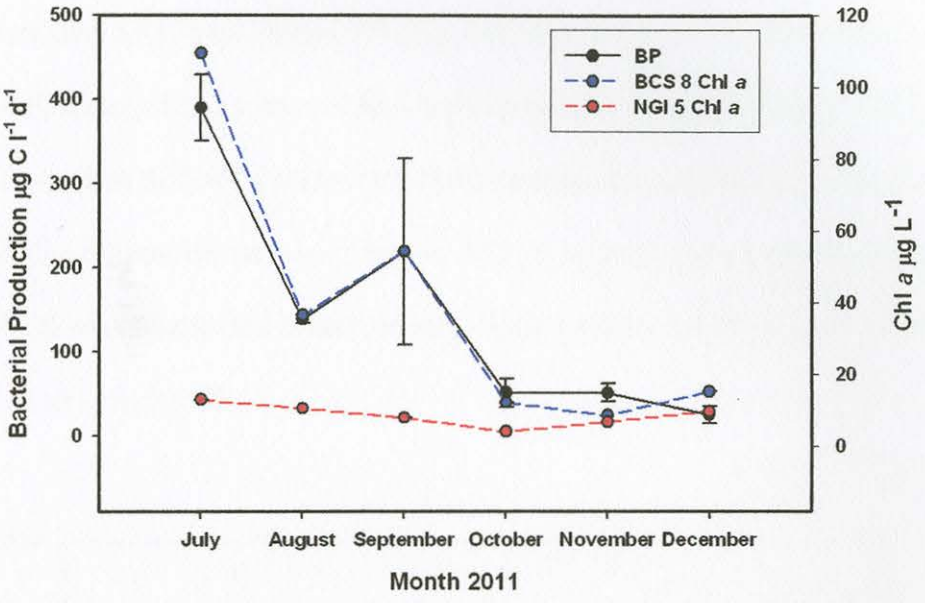


Figure 6. BP versus chl *a* concentration between stations. Using Spearman's rank correlation analysis BP was significantly correlated with chl *a* concentration at BCS 8 ($p<0.05$), but was not correlated significantly with chl *a* at NGI 5 ($p>0.05$).

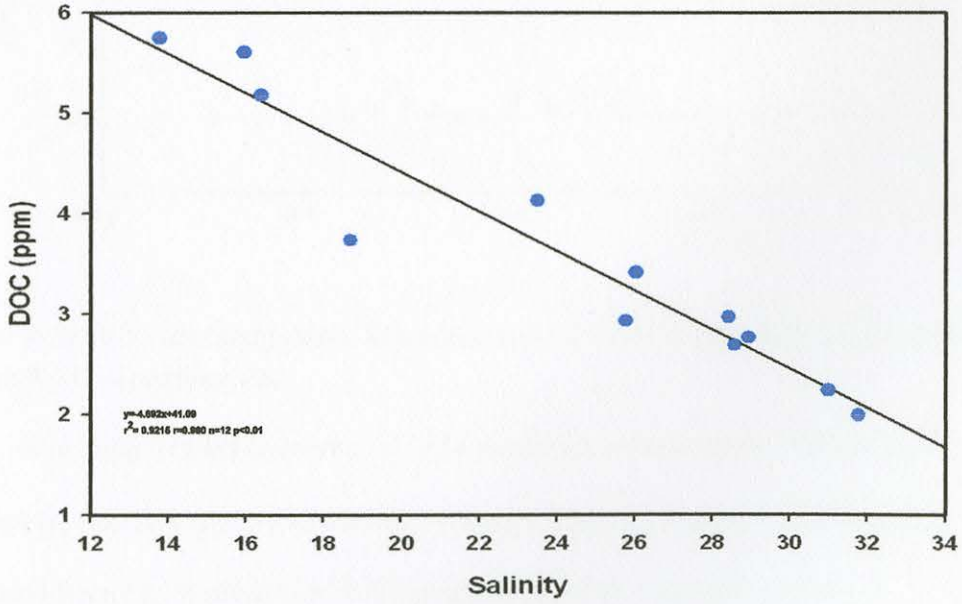


Figure 7. DOC and salinity linear regression. This relationship demonstrates the changes in DOC concentration that resulted along the salinity gradient from BCS 8 to NGI 5.

All of these correlations appear to indicate that silicate is highly affected by freshwater input. There was not a significant difference in DIN and PO_4 temporally or between stations, indicating a fairly static nutrient field during the sampling period. The only other variables that differed significantly between stations were salinity, CDOM, DOC, and TDN. The highest values were found at BCS 8, indicating freshwater influences. In general, DOC was distributed conservatively along a salinity gradient from inshore to offshore waters (Figure 7).

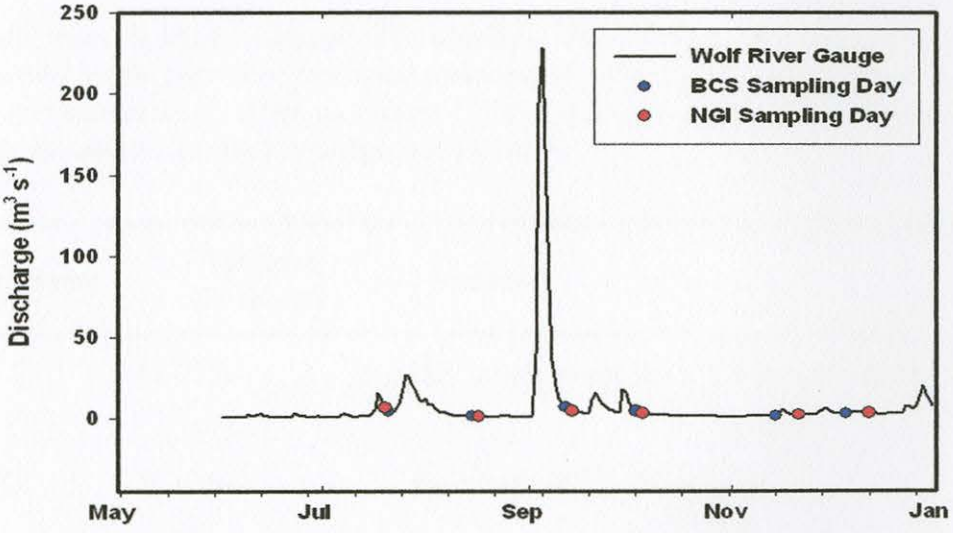


Figure 8. Wolf River Gauge data. Blue dots represent BCS sampling day and red dots represent NGI sampling day.

The study period consisted of little variation in freshwater outflow, except in September, due to a rain event, there was a significant increase in freshwater discharge measured from the Wolf River (WR) gauge (Figure 8). September flow rate was 13.50 $m^3 s^{-1}$ compared to a median of 2.85 $m^3 s^{-1}$ for the entire sampling period. As expected, this increase in discharge was reflected with an increase in BP, chl *a*, DOC, TDN, $Si(OH)_4$, and a decrease in salinity at the inshore site (BCS 8).

Principle Component Analysis

To better explain variability within the study environment, a PCA was completed. The PCA used a 10 variable dataset. Variables that were correlated significantly with multiple variables were omitted (Table 5). The first principle component scores could represent BP and associated variables. The second principle component could be categorized as fluvial inputs and the third principle component could represent the climatological variability.

Table 5

Results from the PCA of dataset (10 variables). 76.01% of the total variance was explained by the first three principal components. Variables omitted from PCA, due to high correlations with other variables: $Si(OH)_4$, CDOM, TDN, air temperature, WR gauge height and monthly average precipitation.

Component	Variance Explained	Variables
1. PC1	41.22%	BP, *in situ* temp., DIN*, 5 day total precip.
2. PC2	20.11%	Salinity*, DOC, chl *a*
3. PC3	14.67%	WR discharge*, PO_4, WSPD*

* DIN, salinity, WR discharge and wind speed were negatively correlated with PC1, PC2, and PC3, respectively.

PER Addition Experiment

Bacterial production rates did not differ significantly between samples augmented with PER and non-augmented water samples (KW, $p>0.05$). The only months that augmented water sample BP rates exceeded non-augmented water samples were in July at NGI 5 (both diatom and chlorophyte PER additions) and August at BCS 8 (chlorophyte PER addition; Figures 9-10). In addition, there were no significant differences in BP when

enhanced by diatom or chlorophyte PER (MW, $p>0.05$; Table 3.6). The median of BP rates for diatom and chlorophyte treatments in the summer season were 195.40 μg C l^{-1} d^{-1}, 210.00 μg C l^{-1} d^{-1}, respectively (Figure 11). In the fall season, median BP rates for diatom and chlorophyte treatments were 31.10 μg C l^{-1} d^{-1}, 29.11 μg C l^{-1} d^{-1}, respectively (Figure 11). The DOC content for the diatom and chlorophyte filtrate was 2,050 μM and 2,375 μM, respectively. Interestingly, this was 5-10 times higher than the DOC in the original water samples, which were in the range of 165.00 μM to 479.17 μM. There still was no significant positive response to the PER additions, indicating waters in the western MS are not limited by labile dissolved organic matter.

Table 6

Kruskall-Wallis analysis of variability between BP in natural water samples compared to samples augmented with phytoplankton exudates. The results indicated no significant different between natural or augmented samples.

Parameter	H	df	Significance
1. Bacterial Production	0.77	2	0.681

Table 7

MW analysis of variability between BP in samples augmented with diatom PER versus chlorophyte PER. The results indicated no significant difference in BP between the two treatments.

Parameter	U	Significance
1. Bacterial Production	66.00	0.729

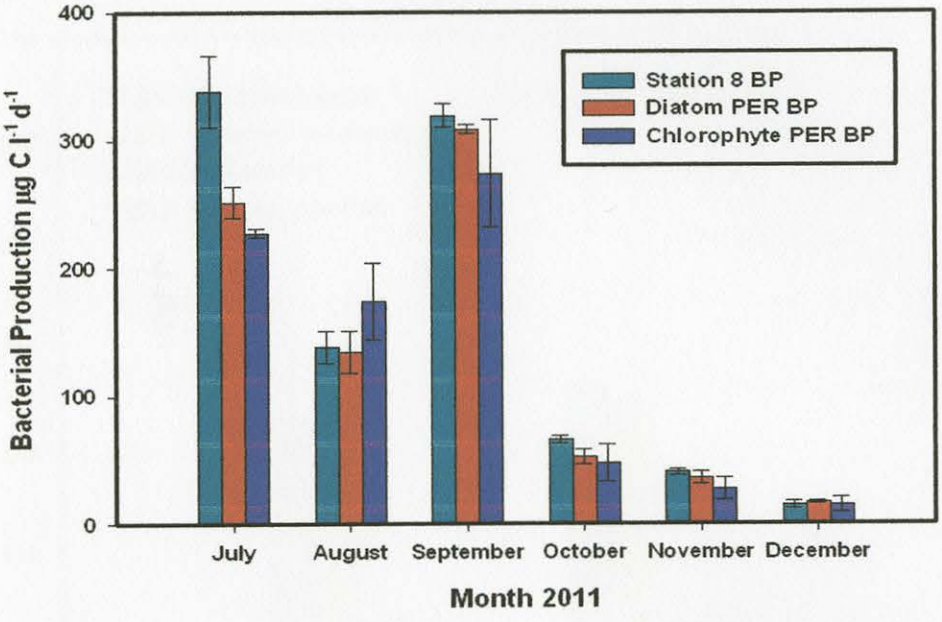


Figure 9. BCS 8 BP rates between PER addition treatments. KW analysis showed no significant difference between treatments ($p>0.05$).

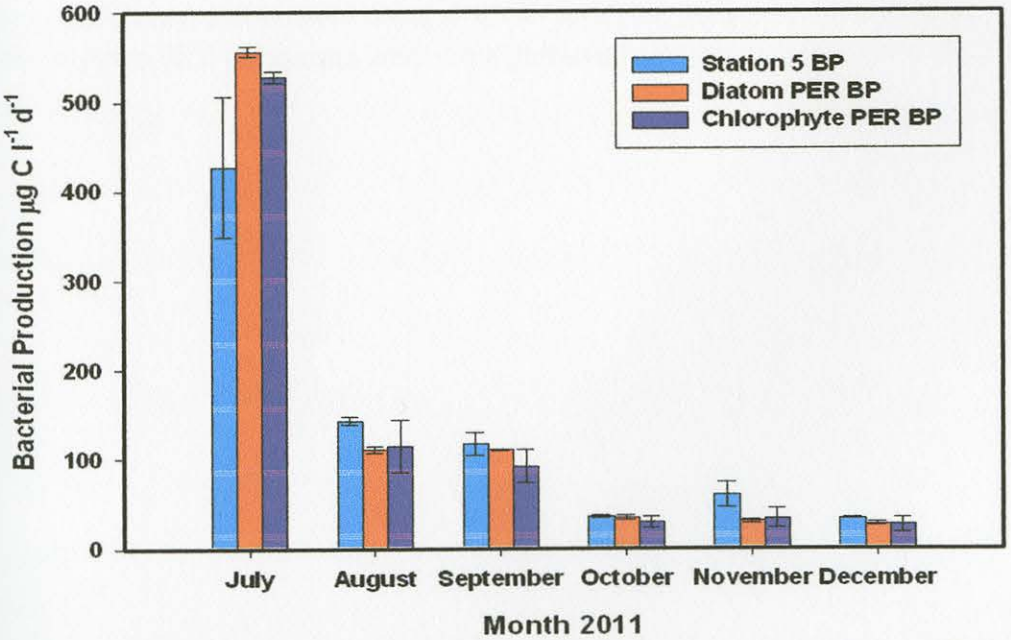


Figure 10. NGI 5 BP rates and PER addition treatments. No significant differences were found between treatments ($p>0.05$, KW).

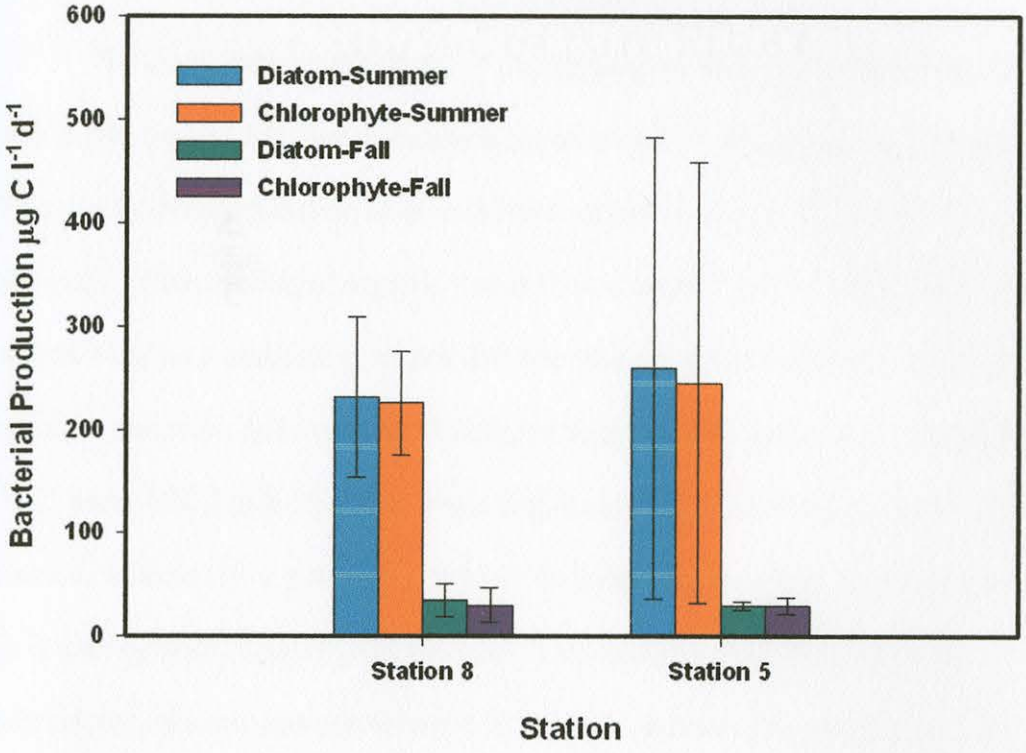


Figure 11. Bacterial production rates for diatom and chlorophyte PER treatments between season and station. Using the MW analysis, differences between diatom and chlorophyte PER treatments were not significantly different from each other ($p>0.05$).

CHAPTER IV

DISCUSSION

The main purpose of this study was to not only investigate BP at two separate sites in the western MS, but to examine the effect PER addition had on BP values. Bacterioplankton production is an important component in coastal marine ecosystems, especially in the cycling of organic matter (Fuhrman and Azam 1980). The results of this project were very interesting, in that, BP rates did not differ between stations. The only variables that were different significantly between stations were chl *a*, salinity, CDOM, DOC, and TDN (Table 2). There was a significant difference between summer and fall seasons, reflected by a positive correlation between *in situ* temperature and BP (*Figure* 5). Bacterioplankton production was also correlated positively with chl *a*, air temperature, silicate, and precipitation. Phosphate and nitrogen species were not correlated significantly with BP. In addition, BP did not differ between PER augmented samples and natural water samples as well as between the PER derived from each of the phytoplankton species used. Bacterial production in the western Mississippi Sound ranged from 14 μg C l^{-1} d^{-1} to 425 μg C l^{-1} d^{-1} (Figure 4.). This is comparable to a literature review of studies conducted in coastal and estuarine systems (range: 0.024 to 1,320 μg C l^{-1} d^{-1}; Apple et al. 2008). Bacterial production rates from other studies in the Mississippi River plume area and open Gulf waters ranged from 2.4 μg C l^{-1} d^{-1} to 273 μg C l^{-1} d^{-1}. Based on these studies, the western Mississippi Sound has the highest BP recorded in the Gulf of Mexico region.

Evaluation of Hypotheses

Hypothesis I

Bacterial production will be greater at station 8 (inshore) compared with station 5 (offshore).

This hypothesis was based on the inshore site being in close proximity to freshwater input with increased nutrients and DOC available for bacterioplankton growth and production. However, based on results from the MW analysis of spatial variability, BP did not differ significantly between stations (Table 2). The variables that differed significantly between stations were chl *a*, salinity, DOC, CDOM, and TDN (Table 2). Higher values were found at the inshore station based on greater freshwater input. Evidence of this can be seen in the PC2 (Table 5) with salinity having the highest loading in the component. Surprisingly, even with greater values of chl *a*, DOC, and nutrient concentrations at BCS 8, BP values were not significantly different between the stations (Table 2).

Murrell (2003) described bacterioplankton dynamics in Pensacola Bay, a subtropical estuary. Bacterial production values averaged 115 μg C l^{-1} d^{-1} (range: 20 μg C l^{-1} d^{-1} to 273 μg C l^{-1} d^{-1}). A strong seasonal signal was apparent with peaks in productivity during summer months. The most productive sites were in the upper estuary, near the mouth of the Escambia River, with high chl *a* concentrations. This was supported by strong positive correlations found between bacterioplankton and chl *a* concentration. Due to low DOC concentrations (range 233.33 μM – 508.33 μM) and POC:PON ratios, Murrell (2003) suggested that Pensacola Bay had relatively low

allochthonous carbon inputs and bacterioplankton were probably driven by phytoplankton production.

A similar pattern was observed in this study, with a strong seasonal signal and peaks of productivity during summer months. The median chl *a* concentration for the entire dataset was 11.33 $\mu g\ l^{-1}$. The inshore median was 25.85 $\mu g\ l^{-1}$ compared to 9.00 $\mu g\ l^{-1}$ at the offshore station. Although the inshore concentrations were higher than offshore, the chl *a* concentrations at both stations were higher than the average (7.0 $\mu g\ l^{-1}$) found in Murrell's study (2003) and exceeded the average of of 7.8 $\mu g\ l^{-1}$ compiled from published studies conducted in coastal and estuarine systems (Apple 2008). The DOC range from this study (165.00 µM – 479.17 µM) was comparable to Murrell's study (2003), suggesting phytoplankton production as the main substrate for bacterioplankton growth at both stations.

Many other studies have found increased BP at sites near freshwater outflows as well as a strong seasonal signal (Almeida et al. 2001; Goosen et al. 1997; McManus et al. 2004; Shiah et al. 1994). These areas were also characterized by increased chl *a* near freshwater dishcharge and during summer months. This is in general agreement with the relationship between bacterioplankton productivity and phytoplankton biomass (Cole et al. 1988). Those results were similar to this study in that higher chl *a* concentrations were found inshore along with a significant BP correlation, but, there was still no increased BP inshore compared to offshore. Almeida et al. (2001) studied factors that influenced bacterial production in Ria de Aveiro, a shallow estuarine system. The results of that study found increased BP in the surface layer but no significant correlation with primary production. Almeida et al. (2001) suggested that even though the phytoplankton

distribution could not explain directly the higher bacterial productivity in the surface layer, irradiation may have enhanced phytoplankton exudation. This would then have caused increased bacterial activity. At NGI 5, surface light intensity was found to increase in this study (Ryan Vandermeulen, personal communication), suggesting that irradiance enhanced exudates may have been a factor. Even though phytoplankton biomass offshore was not as high as inshore phytoplankton biomass, increased irradiation may have caused PER to then be comparable to inshore PER. This may be another reason why offshore BP was not significantly different from inshore BP values.

Coffin et al. (1993) demonstrated that a small labile fraction of the total DOC pool typically supports bacterial growth by examining oxygen consumption and bacterial growth in incubation experiments. Approximately 1% to 3% of the DOC pool was found to support bacterial growth. Also, when labile compounds (algal exudates) were available, greater bacterial activity occurred. Coffin et al. (1993) concluded that bacteria grow most efficiently on labile organic matter and that the quality of organic matter seems to be more important than quantity. This suggests that even though chl *a* concentrations were lower at the offshore station, bacterioplankton were still receiving a sufficient amount of DOM from photosynthetic processes to generate similar BP rates at both sites.

The ratio of BP:PP (bacterial production to primary production) can be considered to be an indicator of the trophic state of a system. Primary production values were calculated for NGI 5 and BCS 8 for the 2011 year (Vandermeulen 2012). The average primary production values from July to December were 0.31 mg O^2 l^{-1} h^{-1} at BCS 8. Primary production rates were only calculated from August to November at NGI

5 with an average of 0.07 mg O_2 l^{-1} h^{-1}. These results were converted to μg C l^{-1} d^{-1} by first converting mg O_2 l^{-1} h^{-1} to moles of O_2 l^{-1} h^{-1} then using the molar ratio of C to O_2 (0.375) to get primary production in moles of C l^{-1} h^{-1}. This was then converted to μg C l^{-1} d^{-1} to be compared with BP rates. The average BP:PP ratio at BCS 8 and NGI 5 was 0.20 and 0.36, respectively. Values having a low (<1) BP:PP ratio demonstrates that PP exceeds BP and indicates an autotrophic system (Goosen 1997). These low BP:PP ratios at both sites show that autochthonous sources of phytoplankton origin keep up with bacterial demand and actually exceed it, leaving surplus DOC for growth or export during summer through fall (McManus et al. 2004).

Temperature is said to play an important role in regulating the composition, abundance and activity of planktonic microbes (Apple et al. 2006). Temperature had a significant correlation with BP at both stations (Table 4). Water temperature ranged near 30° during summer, to 18° in fall, with *in situ* temperature and BP having the two highest loadings in PC1. This corresponds with the strong temperature dependence of heterotrophic microbial processes in estuarine ecosystems (Apple 2008). Temperature has provided the strongest correlation with bacterial abundance and productivity in multiple studies (McManus et al. 2004; Murrell 2003; Shiah 1994). McManus et al. (2004) measured bacterioplankton abundance and productivity in a river-dominated estuary in Mobile Bay. Bacterial activity slowed in winter with low *in situ* temperatures and as temperatures exceeded 15° bacterial abundance increased rapidly. Their results were similar to results of this study, BP at both sites increased when temperatures approached 30° in summer and decreased during fall months. Since similar temperatures

were found between stations, temperature was also a dominate factor contributing to similar BP rates inshore and offshore.

Heterotrophic bacteria utilize inorganic nutrients directly and compete successfully for these materials with phytoplankton (Wheeler and Kirchman 1986; Caron 1994; Kirchman 1994; Hoch and Kirchman 1995). Inorganic nutrients were not found to be correlated significantly with BP from Spearman's rank analysis, except for $Si(OH)_4$ (Table 4). Silicate was also correlated significantly with chl *a*, *in situ* temperature, CDOM, TDN, rainfall, and WR gauge height, and was correlated negatively with salinity. This indicated that the source of silicate in the MS was mainly from freshwater input. Since silicate was correlated significantly with chl *a*, this could be an indicator of diatoms, that need $Si(OH)_4$ to build their frustules. In addition, this may provide a potential link between bacterial production and diatoms in the environment. Principle Component 3 involved PO_4 having a negative relationship with WR discharge and wind speed. This suggested that the source of PO_4 was from the MS rather than from freshwater influence. Dornback (2011) observed the same result, with PO_4 correlated negatively to Pearl and Wolf River outflows. Sawant (2009) studied environmental quality in Bay St. Louis and found the highest PO_4 concentrations at the mouth adjacent to the MS. The MS was found to be an important source of PO_4 to the bay. Although many studies have suggested PO_4 to be an important factor influencing bacterioplankton (Cotner et al. 2000; Pomeroy et al. 1995; Rowe 2008), no significant correlation between the two was found in this study.

From the PCA of the total dataset, BP and DIN contributed to PC1. A negative relationship was found between DIN and BP (Table 5). This relationship confirms that

bacteria consume carbon as a source of energy while scavenging nitrogen for protein synthesis (Azam 1983). Bacteria have a large surface to volume ratio and are adapted to scavenging nutrients from water at very low concentrations (Azam 1983). The DIN and PO_4 nutrient field was rather constant throughout the sampling period. Larsson and Hagstrom (1982) suggested that a static nutrient field forced algae to grow under nutrient limitation, causing increased exudation. Bacterioplankton have the capability to utilize this DOC quickly (Larsson and Hagstrom 1982). Carpenter (2010) conducted experiments on the utilization of inorganic nutrients by bacteria in Mississippi coastal waters. She found no response by microbes to an addition of inorganic nutrients. This suggested that heterotrophic bacteria in Mississippi Coastal waters were not limited by inorganic nutrients. In this study, there was no significant difference in nutrient concentrations and BP between sites (Table 2). This suggested there were sufficient inorganic nutrients available to support similar BP values at both sites studied.

There was little variation in freshwater flow throughout the study period, except for one distinct spike at BCS 8 in September. This led to increased BP, chl *a*, DOC, TDN, $Si(OH)_4$ and decreased salinity. This was due to Tropical Storm Lee that carried heavy rain to the northeastern U.S. in early September. No large increase in PP was found in September (Vandermuelen, 2012), suggesting that organic matter of allochthonous origin played a larger role as a substrate source for BP inshore during September. Freshwater influence and suspended material (measured as turbidity) may have increased the amount of bacteria and phytoplankton in the water samples leading to an overestimation of BP and chl *a* at BCS 8 compared to NGI 5. Bacterioplankton production was also not correlated significantly with DOC and salinity (Appendix B).

Rapid decomposition of fresh DOC may explain why, despite increased in freshwater flow or primary production, no correlations of DOC and bacterial production were found during the six-month sampling period.

Chlorophyll *a* was correlated significantly with BP and BP:PP ratios were <1 at both sites. These results are interesting because BP:PP is often high (>1) in estuaries due to high organic carbon and nutrient concentrations from river and waste input (Goosen et al. 1997). The BP:PP ratios found in the Hudson River estuary and in the Schelde estuary were >1 and a weak correlation was found between bacterial and phytoplankton biomass (Hoch & Kirchman 1993, Goosen et al. 1997). Instead of allochthonous DOM input supporting bacterioplankton growth, phytoplankton extracellular release of DOM seemed to represent a critical substrate source for bacterioplankton metabolism inshore and offshore in western MS. Of the physical factors, temperature was the dominating variable correlated with seasonal variability in BP, in agreement with other studies conducted in estuarine and freshwater systems (White et al. 1991, Shiah, 1993). The results indicate that the western MS had adequate labile substrate, nutrients, and suitable *in situ* temperature to result in similar BP rates at both sites from July to December.

Hypothesis II

Water samples that were augmented with PER will have higher bacterial production compared to non-augmented water samples.

The KW analysis between treatments did not show a significant difference between natural water samples and samples augmented with either diatom or chlorophyte PER (Table 6). Multiple studies have shown that bacteria preferentially utilized DOC of phytoplankton origin (Carpenter 2010; Chen and Wengersky 1996;

Chin-Leo and Benner 1992; Kritzberg 2004). The DOC content for the diatom and chlorophyte filtrate was 2,050 μM and 2,375 μM, respectively. This was 5-10 times greater than the DOC in the original water samples. Surprisingly, the addition of DOC from phytoplankton origin did not have any effect on BP. As discussed earlier, BP in the western MS apparently had more than enough DOC of phytoplankton origin to support observed bacterioplankton growth. Based on chl *a* concentrations and BP:PP ratios, phytoplankton production actually exceeded BP, implying bacteria were feeding essentially on phytoplankton exudates (Petrucia and Barbosa 2004). Kinetics of glucose and amino acid uptake by attached and free-living marine bacteria were compared in the north-western Mediterranean Sea (Ayo et al. 2001). Using the Michaelis-Menten equation, concentration of substrate needed to obtain 90% of V_{max} was estimated. Saturation was reached in seawater at concentrations of substrate ranging from 0.3 μM to 3 μM for amino acids and 0.4 μM to 2 μM for glucose. Although no uptake experiments were done in this study, the saturating concentrations described above may be comparable to those in the western MS because any additional labile DOC did not have an effect on BP, suggesting that the bacteria were near or at saturation with respect to DOM.

Shiah and Ducklow (1994) performed substrate enrichment and temperature-substrate interaction experiments in Chesapeake Bay. Overall, substrate addition did not have a strong effect on bacterial properties, indicating bacteria were not substrate limited. In June and September, substrate addition affected BP when water temperature exceeded 20°C. Similar to the results of Shiah and Ducklow (1994), the only months that augmented water sample BP rates exceeded non-augmented water samples were July

(offshore) and August (inshore) in this study. These months had the highest *in situ* temperature and BP rates. Higher temperatures may enhance multiple chromosome replication and, thus, the rate of cell division (Shiah and Ducklow 1994), an indicator of why the highest BP rates were in the summer season. Shiah and Ducklow (1994) suggested that when temperatures exceed 20°, substrates could be exhausted by fast-growing bacteria and begin to limit bacterial growth. Chin-Leo and Benner (1992) found that in the Mississippi River plume, when temperature exceeded 28°C, bacterial growth was limited by labile carbon. The average DOC concentration offshore in July was 244.17 µM and 284.16 µM inshore during August. These concentrations were lower than the overall DOC average from July to December of 333.33 µM. Although statistics indicated no overall significant difference in BP between treatments, July (offshore) and August (inshore) augmented water samples had higher BP values than the control treatment (Figures 9, 10). This could potentially mean that bacteria were limited by labile DOC in July and September. Higher temperatures allowed for faster maximum bacterial growth rates, which in turn, caused cells to require greater rates of substrate supply.

Hypothesis III

Water samples augmented with diatom PER will have higher bacterial production rates compared to water samples augmented with chlorophyte PER.

This was hypothesized because diatoms are the largest, most abundant phytoplankton species and have high rates of extracellular release of DOM (Granum 2002). They have also been shown to be the predominating species year round in the western MS (Molina 2011). Chlorophytes tend to be smaller and were only found to be

abundant during summer months in the western MS (Molina 2011). The MW test between the two PER addition treatments showed no significant differences in BP when enhanced by diatom or chlorophyte PER (Table 7). As previously discussed, the western MS waters were not limited by labile DOC, so any addition of photosynthetic PER, whether it was diatom or chlorophyte derived would not stimulate BP. On the other hand, bacterial uptake depends on the kind of PER released and the bacterial species involved. Wolter (1982) reported that when *Gymnodinium sp.*, *Skeletonema costatum* and nanoflagellates were present, the highest percentage of exudates were incorporated by bacteria. When a strong bloom of *Chaetoceros* was present, only 3.5% of the exudates were incorporated. Although different percentage of exudates were incorporated by bacteria depending on phytoplankton species, this study's results were interesting because the bacterial community in the western MS did not respond to either diatom or chlorophyte PER addition.

Unexpectedly, it was found that DOC content for the chlorophyte filtrate exceeded diatom DOC filtrate content (2,375 μM to 2,050 μM). Malinsky-Rushansky and Legrand (1996) found PER percentage was higher (12.4%) from a Chlorella-like species compared to *Chaetoceros affinis* with a PER percentage of 9%. *Chlorella vulgaris* had a PER value of 4% and *Skeletonema costatum* had a PER % in the 4.6-9 range (Zlotnik & Dubinsjy 1989, Ignatiades & Fogg 1973). Smaller algae produced more PER, possibly because of their generally higher metabolic rate (Reynolds 1984). While PER was higher for the smaller algae, evidence indicated that bacteria assimilated more PER from larger phytoplankton for biomass, while bacteria taking up PER from smaller phytoplankton had the highest respiration rates (Malinsky-Rushanksky and

Legrand 1996). It would be interesting to continue researching bacterial uptake of PER from these two species based on the surprising results and the important potential roles they play in carbon fluxes through the microbial loop.

Conclusions

The purpose of this study was to investigate BP in the western MS as well as determine the effects PER had on stimulating BP. Bacterial production values were high compared to values measured near the MS, but similar to values reported from estuaries elsewhere. The highest values were reported in July and August with temperature being an important factor for explaining BP seasonal variation. The BP rates found in this study area can provide valuable information for environmental managers, especially during summer months. Since BP is comparatively high, heterotrophic bacteria, both in terms of production and carbon flux, might have a greater trophic importance in this estuarine ecosystem than what was known before.

Bacterial production was not found to vary spatially between the inshore and offshore site. This was due to a constant supply of labile organic matter and inorganic nutrients available at both stations to support bacterial production. The results showed that autochthonous rather than allochthonous sources keep up with bacterial demand during July through December, with an exception in September due to Tropical Storm Lee. This indicated phytoplankton played an important role in the regulating mechanisms of the microbial loop in the western Mississippi Sound during summer/fall months. The addition of PER to natural water samples showed no significant BP stimulation at either site or between phytoplankton species. In summary, the results suggested that temperature and substrate supply affect bacteria interactively and

heterotrophic bacteria in the western MS did not appear to be limited by inorganic nutrients or labile DOC substrates for bacterial production.

APPENDIX A

BACTERIOPLANKTON PRODUCTION DATASET

Bacterial Production (μg C l^{-1} d^{-1}), Chlorophyll *a*, Inorganic and Organic Nutrients, and Environmental Parameters.
ND: No data.

Date	Station	BP	Std. Dev.	Chl *a* (μg l^{-1})	DIN (μM)	NO_3+NO_2 (μM)	NH_4 (μM)	NO_2 (μM)	NO_3 (μM)	Silicate (μM)	PO_4 (μM)
7/21/2011	8	354.014	27.820	109.333	0.481	0.154	0.327	-0.033	0.187	86.918	1.203
7/20/2011	5	425.415	63.920	12.958	0.229	0.229	0.000	0.032	0.197	49.616	0.065
8/15/2011	8	133.210	9.836	36.481	2.540	0.652	1.888	0.248	0.403	63.592	2.051
8/17/2011	5	143.273	4.095	10.547	0.324	0.274	0.050	0.029	0.245	34.059	1.220
9/12/2011	8	319.205	13.264	54.199	1.144	0.195	0.949	-0.034	0.229	95.997	0.630
9/14/2011	5	117.636	12.977	7.894	0.339	0.240	0.099	0.018	0.222	61.838	0.516
10/3/2011	8	65.830	2.468	12.112	1.222	0.161	1.061	-0.042	0.203	74.792	1.038
10/5/2011	5	35.158	2.286	4.018	1.416	0.166	1.25	-0.025	0.191	11.197	0.172
11/14/2011	8	40.496	2.154	8.484	0.314	-0.027	0.341	-0.061	0.034	0.940	0.940
11/21/2011	5	59.966	13.711	6.797	0.765	0.024	0.741	-0.031	0.055	2.058	0.042
12/5/2011	8	14.798	3.014	15.215	1.258	0.009	1.249	-0.057	0.066	1.095	0.513
12/12/2011	5	33.338	0.706	9.712	1.685	0.399	1.286	0.046	0.353	5.997	0.148

Date	Station	In situ Temp.	Salinity	sigmaT (kg/m^3)	Oxygen mg L^{-1}	CDOM	Resp. (μg O_2 l^{-1} d^{-1})	DOC ppm	TDN ppm
7/21/2011	8	30.580	16.383	1007.650	12.988	37.020	8440.8	5.177	0.363
7/20/2011	5	29.302	25.785	1015.070	14.175	20.640	ND	2.930	0.236
8/15/2011	8	30.003	26.054	1015.040	4.265	29.906	5772	3.412	0.296
8/17/2011	5	29.282	28.947	1017.440	6.028	18.956	2865.6	2.758	0.230
9/12/2011	8	26.256	13.781	1007.030	6.066	56.517	3470.4	5.746	0.402
9/14/2011	5	27.994	18.690	1010.180	7.356	30.822	2376	3.731	0.253
10/3/2011	8	22.847	15.944	1009.590	6.495	56.168	3050.4	5.601	0.340
10/5/2011	5	24.136	28.579	1018.750	5.918	18.650	1761.6	2.682	0.205
11/14/2011	8	17.266	23.508	1016.660	6.976	32.890	5659.2	4.124	0.490
11/21/2011	5	18.286	31.778	1022.740	6.809	12.543	3026.4	1.981	0.203
12/5/2011	8	15.392	28.436	1020.840	7.025	24.583	2841.600	2.966	0.245
12/12/2011	5	14.200	31.000	ND	ND	ND	6760.800	2.229	0.165

Weather (NDBC), precipitation (NCDC), and WR tide gauge data (USGS).

Date	Station	WDIR	WSPD	GST	PRES	ATMP	Monthly Avg.	4d prior	3d prior
7/21/2011	8	SSE	3.590	4.600	1014.990	28.605	13.600	0.720	3.100
7/20/2011	5	SSW	2.748	3.305	1014.918	28.437	13.600	0.000	0.720
8/15/2011	8	W	2.500	3.160	1017.200	29.500	0.920	0.000	0.000
8/17/2011	5	ESE	2.290	2.661	1016.847	29.176	0.920	0.000	0.000
9/12/2011	8	SW	2.550	3.408	1019.841	25.542	7.960	0.000	0.000
9/14/2011	5	SW	4.592	5.243	1015.279	27.123	7.960	0.000	0.000
10/3/2011	8	E	4.056	4.954	1022.430	18.674	0.270	0.370	0.000
10/5/2011	5	ESE	5.714	6.276	1020.971	22.379	0.270	0.000	0.000
11/14/2011	8	SSE	3.473	4.501	1017.020	19.164	2.440	0.140	0.000
11/21/2011	5	ESE	3.556	4.173	1022.595	19.344	2.440	0.000	0.000
12/5/2011	8	SE	4.082	6.040	1016.974	18.249	0.930	0.000	0.000
12/12/2011	5	ENE	5.783	6.570	1026.453	10.467	0.930	0.000	0.000

Date	Station	2d prior	1d prior	Sample day	5d total	WR Gauge Ht	WR Discharge
7/21/2011	8	0.670	0.000	0.000	4.490	1.491	3.940
7/20/2011	5	3.100	0.670	0.000	4.490	1.666	6.262
8/15/2011	8	0.000	0.000	0.220	0.220	1.403	1.220
8/17/2011	5	0.220	0.000	0.000	0.220	1.381	1.330
9/12/2011	8	0.000	0.000	0.000	0.000	2.782	6.970
9/14/2011	5	0.000	0.000	0.000	0.000	1.981	13.490
10/3/2011	8	0.000	0.000	0.000	0.370	1.740	1.840
10/5/2011	5	0.000	0.000	0.000	0.000	1.733	8.657
11/14/2011	8	0.000	0.000	0.000	0.140	1.313	1.420
11/21/2011	5	0.000	0.000	0.000	0.000	1.425	2.710
12/5/2011	8	0.000	0.000	0.100	0.100	1.491	2.690
12/12/2011	5	0.170	0.140	0.000	0.310	1.454	2.990

BP results from samples augmented with PER from diatom and chlorophyte phytoplankton species. D: samples augmented with diatom PER. C: samples augmented with chlorophyte PER.

Month	Station	Species	BP ($\mu g\ C\ l^{-1}\ d^{-1}$)	Std. Dev.
7/21/2011	8	D	251.682	13.480
7/21/2011	8	C	227.608	15.490
7/20/2011	5	D	553.174	37.560
7/20/2011	5	C	524.819	34.720
8/15/2011	8	D	132.812	21.070
8/15/2011	8	C	172.430	45.799
8/17/2011	5	D	110.766	5.010
8/17/2011	5	C	113.433	36.641
9/12/2011	8	D	308.589	17.030
9/12/2011	8	C	273.643	46.384
9/14/2011	5	D	110.236	1.714
9/14/2011	5	C	91.541	27.552
10/3/2011	8	D	52.281	7.995
10/3/2011	8	C	47.564	21.140
10/5/2011	5	D	33.957	1.386
10/5/2011	5	C	28.972	9.268
11/14/2011	8	D	33.782	3.879
11/14/2011	8	C	29.518	16.312
11/21/2011	5	D	29.778	3.319
11/21/2011	5	C	33.273	9.655
12/5/2011	8	D	16.931	0.687
12/5/2011	8	C	14.728	7.428
12/12/2011	5	D	26.642	2.572
12/12/2011	5	C	25.858	11.793

APPENDIX B

SPEARMAN RANK CORRELATIONS AND MW ANALYSIS FOR THE ENTIRE DATASET

Spearman's Rank Correlation for the total dataset. The values in **bold** indicate highly significant correlations (n=12, $p \leq 0.01$). The values in standard font indicate significant correlations ($p \leq 0.05$). Dashed lines indicate values that are not significant. Bacterial production was positively correlated with Chl *a*, *in situ* temperature, silicate and negatively correlated with density.

	Chl *a*	DIN	NH_4	NO_2	NO_3	$Si(OH)_4$	PO_4	*In situ* T	Salinity	Density	DO
BP	**0.673**	---	---	---	---	**0.727**	---	**0.867**	---	-0.636	---
Chl *a*		---	---	---	---	0.601	---	---	---	-0.618	---
DIN			---	---	---	---	---	---	---	---	0.609
NH4				---	---	---	---	---	---	---	---
NO2					---	---	---	---	---	---	---
NO3						---	---	---	---	---	---
SiO3							---	0.692	-0.692	**-0.891**	---
PO4								---	---	---	---
Salinity									---	**0.918**	---
Density										---	---
DO											---

	CDOM	DOC	TDN	WSPD	PRES	ATMP
BP	---	---	---	-0.650	-0.594	**0.790**
Chl *a*	0.609	0.594	---	---	---	---
DIN	---	---	---	---	0.685	-0.580
NH4	---	---	---	---	0.627	---
NO2	---	---	---	---	---	---
NO3	---	---	---	---	---	---
SiO4	0.636	0.629	---	---	---	---
PO4	---	0.580	0.601	---	---	---
DO	---	---	---	---	-0.645	---
In situ T	---	---	---	---	-0.643	---
Salinity	**-0.955**	**-0.965**	**-0.867**	---	---	---
Density	**-0.873**	**-0.873**	**-0.736**	---	---	---
TDN	**0.927**	**0.937**	---	---	---	---

Spearman's Rank Correlations of precipitation and hydrologic data. BP was correlated with 5 day total precipitation (precipitation 4 days before plus sampling day). WR gauge height was correlated positively to SiO_4 and negatively with salinity. The monthly average precipitation was correlated positively with WR discharge.

	Monthly Avg	Sample day	5d total	WR Gauge HT	WR discharge
BP	---	---	0.723	---	---
Chl *a*	---	---	---	---	---
DIN	-0.580	---	---	---	---
NH_4	---	---	---	---	---
NO_2	---	---	---	---	---
NO_3	---	---	---	---	---
$Si(OH)_4$	---	---	0.759	0.636	---
PO_4	---	---	---	---	---
DO	**0.772**	---	---	---	---
In situ T	---	---	---	---	---
Salinity	---	---	---	-0.608	---
Density	---	---	-0.764	---	---
TDN	---	---	---	---	---
Monthly Avg	---	---	---	---	0.678

Spearman's Rank Correlation for BCS 8. The values in **bold** indicate highly significant correlations (n=6, $p \leq 0.01$). The values in standard text indicate significant correlations ($p \leq 0.05$).). Dashed lines indicate values that are not significant. Bacterial production was correlated positively with chl *a*, silicate, and *in situ* temperature. BP and silicate were correlated inversely with density. Salinity was correlated positively with density. NO_2 values were not included due to insufficient data.

	Chl *a*	DIN	NH_4	NO_3	$Si(OH)_4$	PO_4	*In situ* T	Salinity	Density	DO
BP	0.829	---	---	---	0.829	---	**0.943**	---	-0.886	---
Chl *a*		---	---	---	---	---	---	---	---	---
DIN			---	---	---	---	---	---	---	---
NH_4				---	---	---	---	---	---	---
NO_3						---	---	---	---	---
$Si(OH)_4$							---	---	---	**0.943**
PO_4								---	---	---
In situ T									---	---
Salinity										0.886

	CDOM	DOC	TDN	WSPD	PRES	ATMP	Monthly Avg	Sample day	5d total	WR Gauge HT
BP	---	---	---	---	---	---	---	---	0.900	---
Chl *a*	---	---	---	---	---	---	---	---	---	0.841
DIN	---	---	0.886	---	---	---	---	---	---	---
NH_4	---	---	---	---	---	---	---	---	---	---
NO_2	---	---	---	---	---	---	---	---	---	---
NO_3	---	---	---	---	---	---	---	---	---	---
$Si(OH)_4$	---	---	---	---	---	---	---	---	0.900	---
PO_4	---	---	---	---	---	---	---	---	---	---
In situ T	---	---	---	---	---	0.886	---	---	---	---
Salinity	**-1.00**	**-1.00**	---	---	---	---	---	---	---	---
Respiration	---	---	---	---	---	---	---	---	1.00	---
Density	-0.886	0.886	---	---	---	0.886	---	---	---	---
CDOM	---	1.00	---	---	---	---	---	---	---	---

Spearman Rank Correlation of Station 5. The values in **bold** indicate highly significant correlations (n=6, $p \leq 0.01$). The values in standard text indicate significant correlations ($p \leq 0.05$).). Dashed lines indicate values that are not significant. BP is correlated positively with DIN, NH_4, and *in situ* temperature.

	Chl *a*	DIN	NH_4	NO_2	NO_3	$Si(OH)_4$	PO_4	*In situ* T	Salinity	Density	DO
BP	---	-1.00	-1.00	---	---	---	---	**0.943**	---	---	---
Chl *a*	---	---	---	---	---	---	---	---	---	---	---
DIN	-1.00	---	---	---	---	---	---	-0.943	---	---	---
NH_4			---	---	---	---	---	-0.900	---	---	---
NO_2				---	---	---	---	---	---	---	---
NO_3					---	---	---	---	---	---	---
$Si(OH)_4$						---	---	---	-0.943	-1.00	---
PO_4							---	---	---	---	---
In situ T								---	---	---	---
Salinity									---	0.900	---
Density										---	---

	CDOM	DOC	TDN	WSPD	PRES	ATMP	Monthly Avg	Sample day	5d total
BP	---	---	---	-0.886	-0.886	0.886	---	---	---
Chl *a*	---	---	---	---	---	---	---	---	---
DIN	---	---	---	0.886	0.886	-0.886	---	---	---
NH_4	---	---	---	0.900		-0.900	---	---	---
NO_2	---	---	---	---	---	---	---	---	---
NO_3	---	---	---	---	---	---	---	---	---
SiO_4	1.00	1.00	0.943	---	-0.886	---	---	---	---
PO_4	---	---	---	---	---	---	---	---	---
In situ T	---	---	0.829	---	**-0.943**	**0.943**	---	---	---
Salinity	-0.900	**-0.943**	-0.886	---	0.829	---	---	---	---
Density	**-1.00**	**-1.00**	**-1.00**	---	0.900	---	---	---	---
DO	---	**1.00**	**1.00**	---	---	---	---	**1.00**	---
DOC	---	---	---	---	-0.886	---	---	---	---
CDOM	---	---	---	---	-0.900	---	---	---	---
TDN	---	---	---	---	-0.943	---	---	---	---

MW test for differences between stations. N=12. The MW statistic (U) with a significance listed ($p \leq 0.01$ for values in **bold**, $p \leq 0.05$ for values in standard type) indicates that there is a statistically significant difference for that parameter between seasons. Dashed lines indicate no significant difference between stations. For respiration, n=11 and NO_2, n=5.

Parameter	U	Significance
BP	17.000	---
Chl *a*	4.000	0.025
DIN	12.000	---
NH_4	12.000	---
NO_3	15.000	---
NO_2	0.000	---
$Si(OH)_4$	12.000	---
PO_4	6.000	---
In situ T	17.000	---
Salinity	5.000	0.037
Density	7.000	---
Oxygen	14.000	---
CDOM	2.000	0.018
DOC	2.000	**0.010**
TDN	1.000	**0.006**
Respiration	7.000	---

MW test for differences between seasons. N=12. The MW statistic (U) with a significance listed ($p \leq 0.01$ for values in **bold**, $p \leq 0.05$ for values in standard type) indicates that there is a statistically significant difference for that parameter between seasons. Dashed lines indicate no significant difference between stations. For respiration, n=11 and NO_2, n=5.

Parameter	U	Significance
BP	0.000	**0.004**
Chl *a*	7.000	---
DIN	9.000	---
NH_4	8.000	---
NO_3	8.000	---
NO_2	1.000	---
$Si(OH)_4$	4.000	0.025
PO_4	9.000	---
In situ T	0.000	**0.004**
Salinity	10.000	---
Density σ_t	5.000	---
Oxygen	13.000	---
CDOM	11.000	---
DOC	11.000	---
TDN	12.000	---
Respiration	12.000	---

APPENDIX C

PRINCIPAL COMPONENTS ANALYSIS OUTPUT

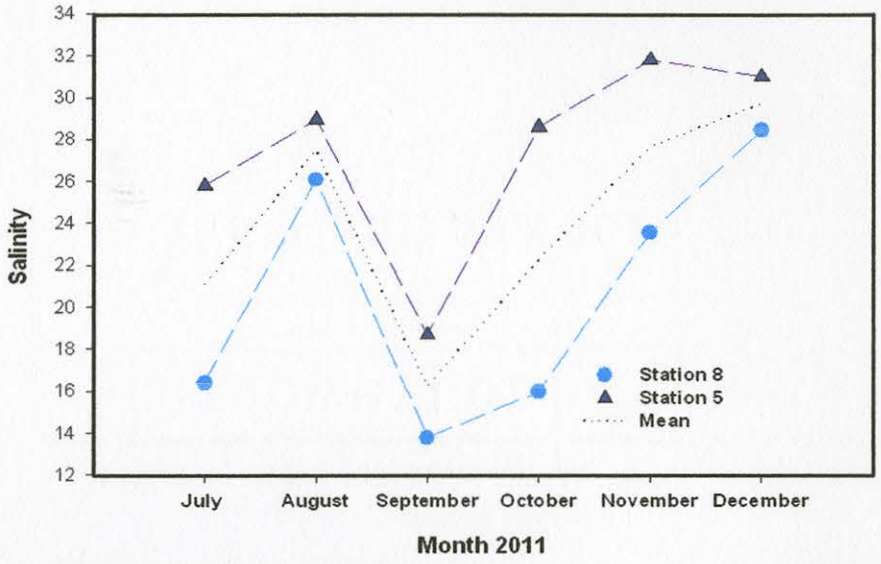
Principle Components Analysis SPSS output for total dataset. PCA extracted multiple components based on a VARIMAX rotated correlation matrix. However, only the first three components were found to be of significant value. Listed below are the components and their associated variables with loading values. The closer the value is to 1, the more variance the variable explains. Variables omitted due to their high correlations with other variables: $Si(OH)_4$, CDOM, TDN, air temperature, WR gauge height and monthly average precipitation.

Component	Variable	Loading Values
PC1	BP	0.890
	5 day total precip.	0.859
	In Situ Temp.	0.637
	DIN	-0.605*
PC2	Salinity	-0.951*
	DOC	0.935
	Chl *a*	0.612
PC3	WR Discharge	-0.821*
	PO_4	0.797
	WSPD	-0.628*

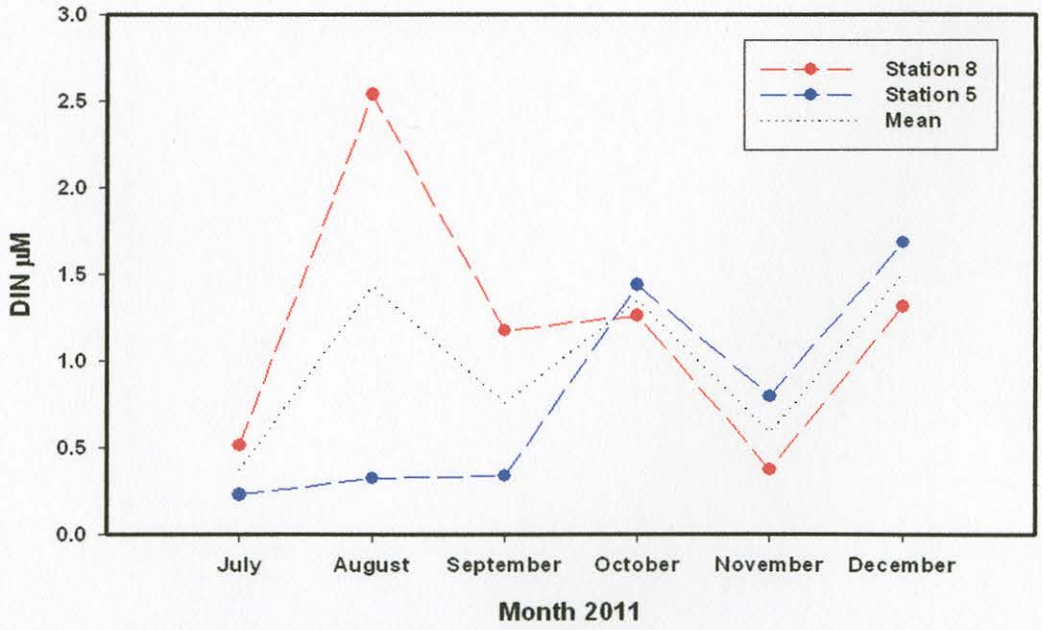
*DIN, Salinity, WR Discharge and WSPD were correlated negatively with other variables on PC1, PC2, and PC3, respectively.

APPENDIX D

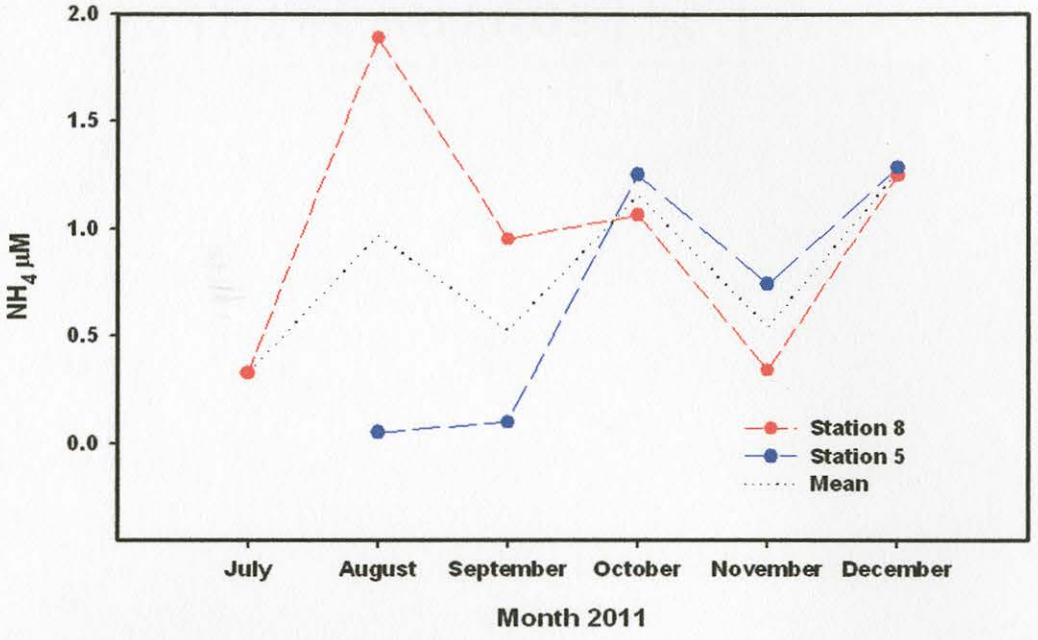
BACTERIOPLANKTON SUPPLEMENTAL FIGURES AND INFORMATION



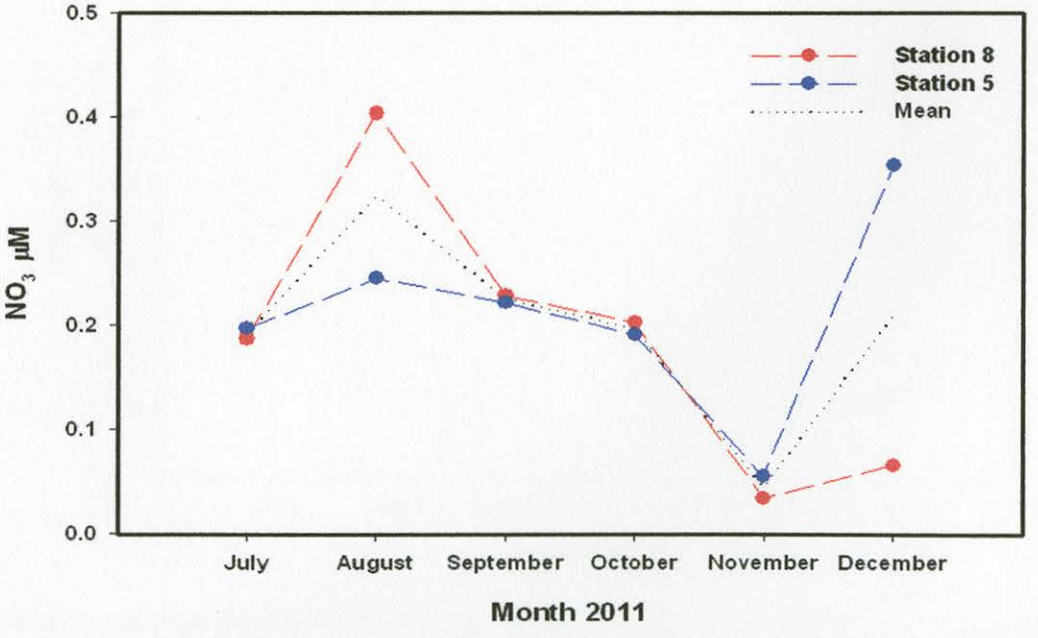
Average salinity from July to December 2011 between station 8 and station 5. Station 8 is represented by the blue line and station 5 is represented by the purple line. Salinity increased from station 8 to station 5 due to proximity from freshwater source.



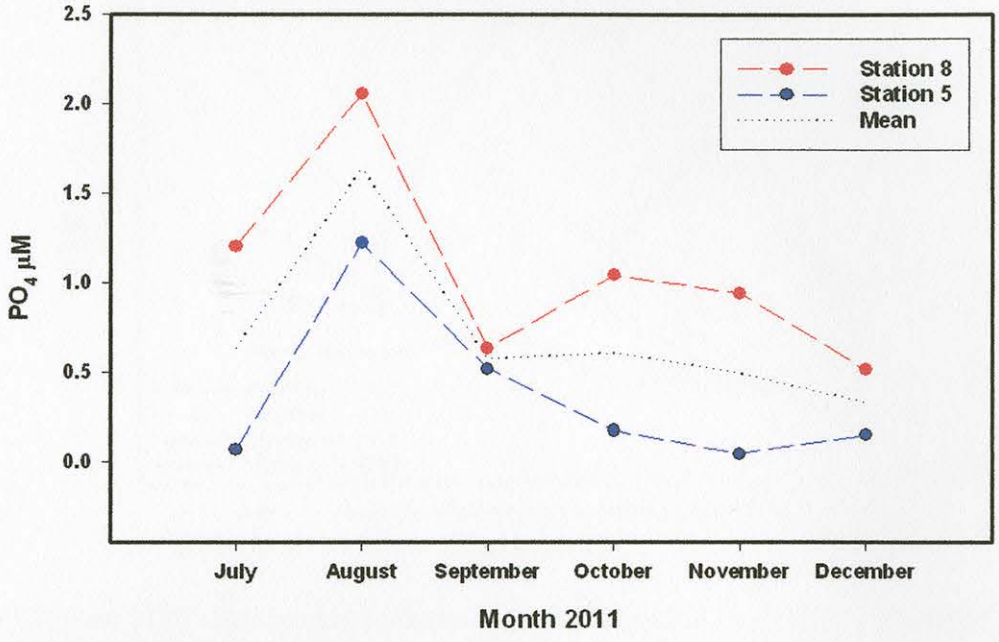
Variation of DIN concentration between stations 8 and 5. The overall mean was 135.80 µM, with a range of 0.23 (July, station 5) to 2.54 µM (September, station 8).



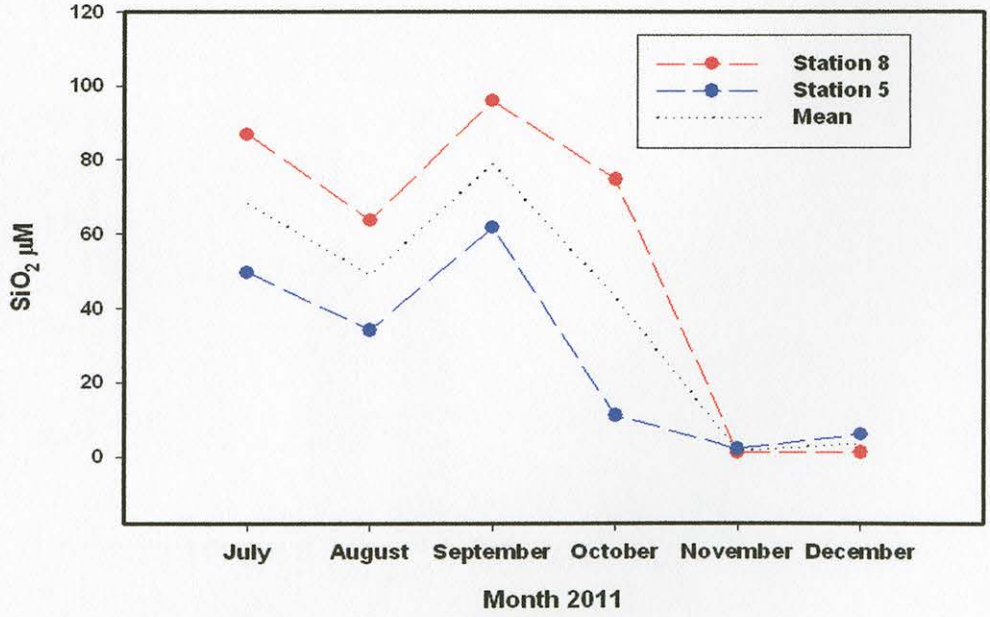
Variation of NH_4 concentration between stations. The overall mean was 0.89 µM, with a range of 0.05 (August, station 5) to 1.89 µM (August, station 8). There was no value in July at station 5, NH_4 could not be detected on the nutrient analyzer.



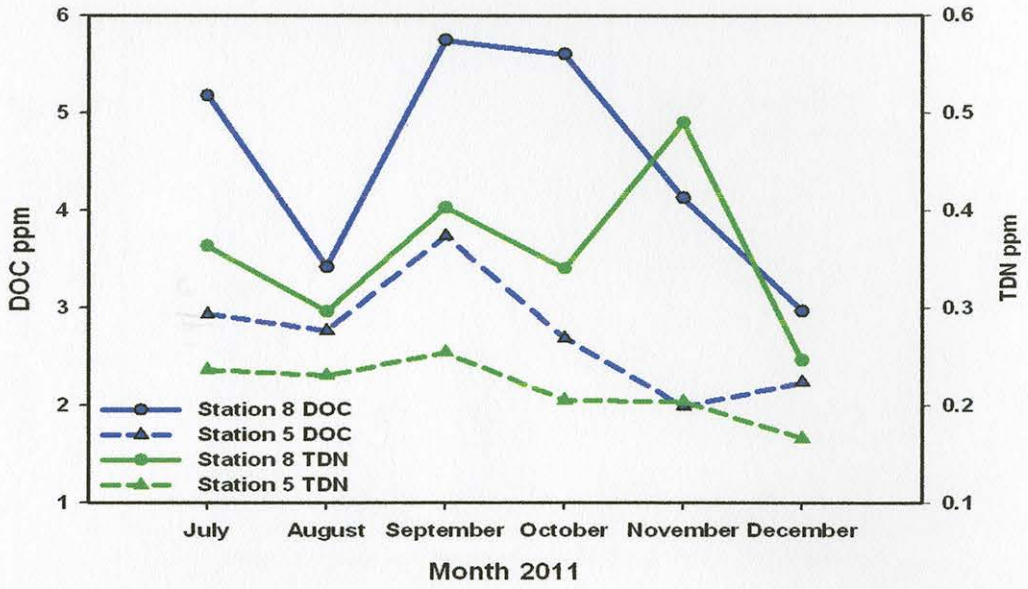
Variation of NO_3 between stations. The overall mean was 0.20 µM, with a range of 0.034 (November, station 8) to 0.403 (August, station 8).



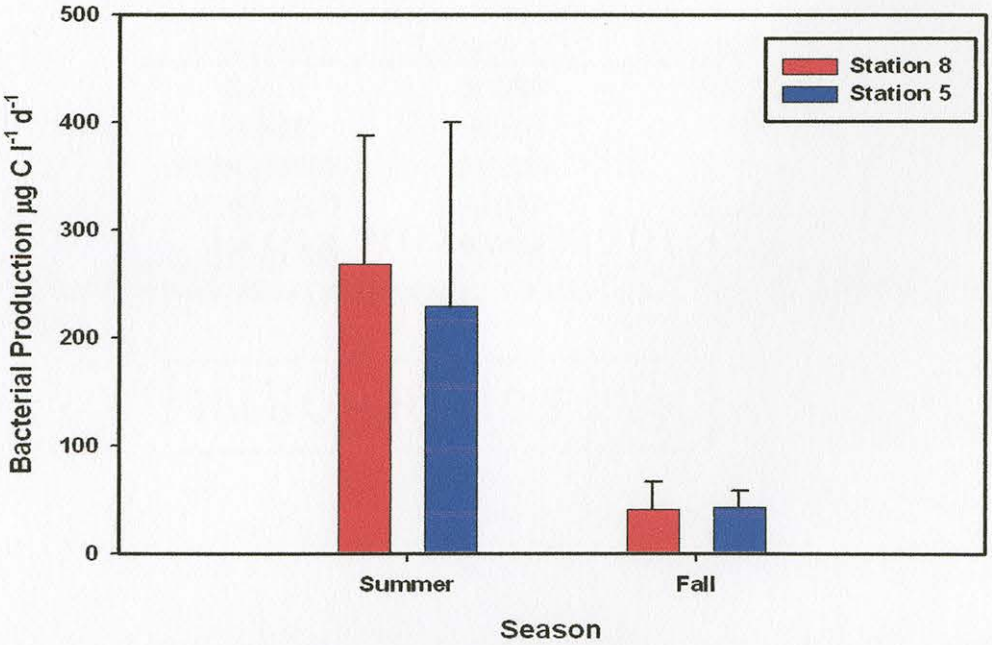
Variation of PO_4 between stations. The overall mean was 0.712 µM, with a range of 0.042 (November, station 5) to 2.05 µM (August, station 8).



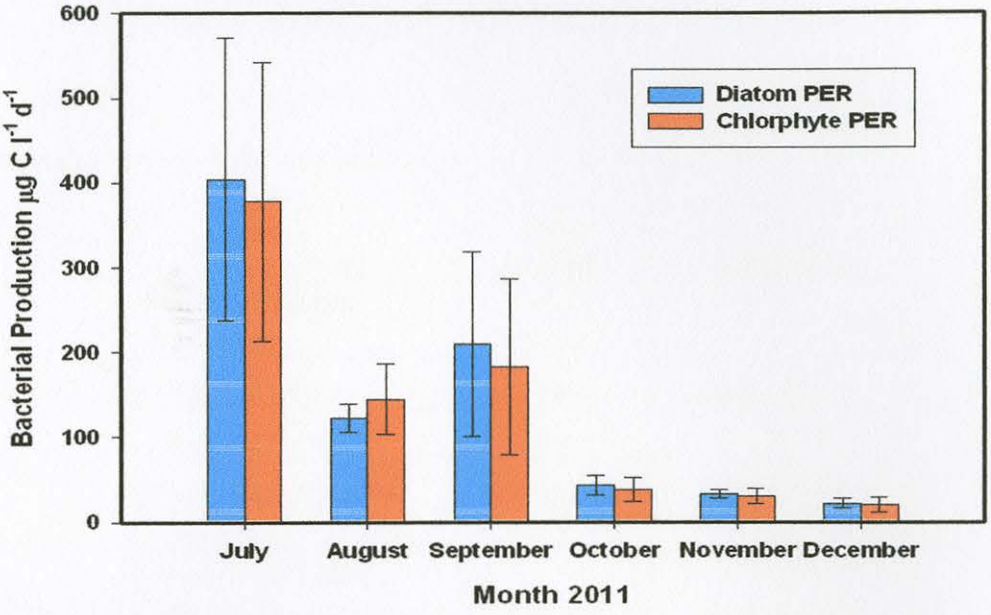
Variation of $Si(OH)_4$ between stations. The overall mean was 40.68 µM, with a range of 0.94 (November, station 8) to 95.99 µM (September, station 8).



DOC and TDN relationship between stations. Blue line represents DOC and green line represents TDN. The circle represents Station 8 and the triangle represents Station 5. Mann-Whitney sum test for spatial variance showed significant difference between DOC and TDN between stations ($p \leq 0.01$).



Bacterial production between summer and fall season. Mann-Whitney analysis results indicate a significant difference in BP between seasons ($p \leq 0.001$).



Bacterial production rates for diatom PER treatment versus chlorophyte PER treatment from July through December.

Study Stations and GPS Coordinates

Station	Latitude (°N)	Longitude (°W)
8-BCS	30.298	-89.263
5-NGI*	30.161	-89.045
NDBC-BWYC	32.326	-89.326
NDBC-GPT	30.230	-88.982
USGS-WR	30.484	-89.274

*Alternative NGI station in December: Latitude (°N): 30.2132, Longitude (°W): -89.032

APPENDIX E

ALGAL CULTURE FORMULA (FCRG MEDIUM)

Stock solution (per Liter of medium)

Compound	Stock Solution	Final Concentration
963 mL Filtered Seawater	-	-
10 mL KNO_3	5.00 g L^{-1}	494.54 µM
10 mL KH_2PO_4	0.68 g L^{-1}	49.97 µM
5 mL Na_2SiO_3 $9H_20$	14.00 g L^{-1}	246.31 µM
5 mL HCL	0.07 N	-
1 mL F/20 Trace Metal Mix	Recipe	-
1 mL F/10 Vitamin Mix	Recipe	-
0.2 g Sodium Bicarbonate	$NaHCO_3$	-

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